



Biotechnology for Sustainable Agriculture

Emerging Approaches and Strategies

Editors
Ram Lakhan Singh
Sukanta Mondal

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Dedication

**Dedicated to
the students and researchers
who refined our knowledge of Biotechnology
by their intelligent questions, queries, and discussions
over the years.**

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Preface

Developing an efficient sustainable agriculture under current context of major global threats (climate change, soil degradation and erosion, water scarcity, biodiversity diminution) coupled with a continual population growth represents an imperative for conceiving a coherent strategy aimed to ensure the food and livelihood security. The term “sustainable agriculture” encompasses novel agricultural methods to protect the environment, conserve natural resources, diminish the use of chemical compounds, and promote financial independence. Despite the advancements in agricultural production following the development of the Green Revolution technologies, the current production rates have failed to meet population needs and the world is facing food issues. Since the world population has been estimated to reach eight billion in 2030, i.e., one billion more than the present population, improving the quality and quantity of food production is an inevitable necessity. Meanwhile, the limited available resources have compelled scientists, food industry stakeholders, and policy makers to seek a solution for providing the basic needs of this growing population. Sustainable livestock production using modern biotechnology is the need of the hour because it contributes more effectively to gains in productivity, reduction of poverty, and become ecologically more sustainable. Current innovative biotechnologies such as recombinant DNA technology and genetic engineering have tremendous potential for impacting global food security, human and animal health, environmental health, and overall livelihood of mankind. The current excitement over biotechnology came from explosive development and understanding of life processes at molecular level, more precise method to manipulate genetic material, the DNA, and to develop full grown animals from genetically modified cells. The recent development in molecular biology and biotechnology resulted in unlimited access to gene pool and enhanced the pace and precision of creating gene sequencing and functional genomics to meet the challenges of food, agriculture, and animal improvement.

It gives us immense pleasure to introduce this book *Biotechnology for Sustainable Agriculture: Emerging Approaches and Strategies*. The present book addresses all the issues for sustainable agriculture and their solutions by way of modern day and future biotechnology. The book begins by laying the fundamentals of biotechnology and sustainable agriculture. It then focuses on a detailed discussion of biotechnological tools to enhance

sustainable production, sustainable agriculture and food security, plant biotechnology and crop improvement, transgenic animal production, microbial biotechnology, and sustainable agriculture. This book also covers the impact of climate change on agriculture, livestock production and fisheries, nanotechnology and its implication in agricultural biotechnology, biosafety for sustainable agriculture, genetic engineering and public perception, and the future course.

The book is easy to follow with simple explanations and a good framework for understanding the role of biotechnology for sustainable agriculture. All chapters have been designed and prepared by the authors in such a way that present the subject in-depth following a student-friendly approach. The contributors to the book are internationally recognized experts in their field and they represent reputed institutions across the globe. This book is valuable resource for graduate and undergraduate students, researchers, instructors and extension experts in agriculture, veterinary, and fishery sciences. Each chapter has a short bibliography which may serve as entry point to the research students.

KEY FEATURES OF THE BOOK

The text of the book includes certain important features to facilitate better understanding of the topics discussed in the chapters.

Abstract has been presented in the beginning of each chapter to highlight the important concepts discussed in the chapter.

Tables and figures dispersed throughout the chapters enable the reader easy understanding of the concepts discussed.

References at the end of each chapter familiarize the readers with important texts and articles cited which may be helpful for further in-depth studies related to the topic.

ORGANIZATION OF THE BOOK

This book consists of 13 chapters that focus on current approaches and strategies for sustainable agricultural/livestock production under normal and climate change scenario.

Chapter 1 traces the brief introduction, scope and applications of biotechnology for sustainable agriculture, and livestock production.

Chapter 2 deals with the biotechnological tools for augmenting agricultural sustainability. Biotechnological tools are processes of bioscientific interests that harness the cellular and biomolecular processes of living organisms to help advance human health, improve agricultural production, animal health and welfare, and provide environmental conservation benefits.

Chapter 3 demonstrates the specialized knowledge, understanding, and skills necessary to contribute effectively and ethically to strategic decision

making, opinion forming, and operational management for the sustainable development of agriculture and food security.

Chapter 4 covers molecular breeding and genetic engineering, and their integration with conventional breeding to develop crops that are more nutritious, resistant to disease, and more tolerant of abiotic stresses. The chapter focuses on development of transgenic crops with desired traits, its advantages and disadvantages, risk and controversies about use of GM crops as well as philosophical and religious concerns.

Chapter 5 includes the state-of-the-art of animal transgenesis, contribution for animal welfare, pitfall and risks, and ethical concerns. Genetically modified (transgenic) livestock, stem cells, and other emerging biotechnologies have important roles in producing better quality as well as quantity food derived from livestock, improved strain of livestock, enhanced prolificacy and reproductive performance, increased feed utilization and growth rate, improved carcass composition, improved milk production and/or composition, modification of hair or fiber, and increased disease resistance.

Chapter 6 focuses on the recent developments in our understanding of the roles of microbes in sustainable agriculture and their diversified applications in agriculture as biofertilizers, bio-pesticides, bioherbicides, etc.

Chapter 7 describes the state of current scientific knowledge on the links between climate change, agriculture and food security in terms of anticipating impacts, managing climate variability and risk, accelerating adaptation to progressive climate change, and mitigating greenhouse gas emission from agriculture sector.

Chapter 8 illustrates temperature humidity index, climate change impact on milk production, estrous cycle, oocyte maturation, and embryo development as well as climate smart strategies (genetic, physical modification of environment, nutritional management) for minimizing the impact of stress for fertility augmentation.

Chapter 9 includes the impact of climate change on ecosystem, aquatic biota, cascading effects of ecosystem production change, conceptual framework for understanding vulnerability, adaptation and resilience, managing climate hazards and adaptation strategies to make fisheries sustainable on the face of global warming, and improve livelihoods of farmers.

Chapter 10 discusses the limits of conventional farming, advantages of nanomaterials, distinctiveness of agricultural production system, nanotechnology in agriculture for livelihood security, socio-economic issues, and public acceptance of nanotechnology.

Chapter 11 deals with biosafety rules, regulations, legislations, risk assessment, risk management, and monitoring mechanisms to ensure the appropriate utilization of the products of modern biotechnology.

Chapter 12 focuses on public perception of genetic engineering, awareness about safety and acceptance of GM foods/animal, relationships between consumers' perception and knowledge about genetically modified organisms

(GMO) and their disposition to the introduction of GM crops and drafting of any national policy on GMO.

Chapter 13 deals with the way ahead for the development of highly productive, efficient, resistant (to biotic and abiotic stresses), remunerative, quality-rich genotypes suitable both for congenial (irrigated) and for noncongenial (rainfed/dryland) settings, which when blended with time-tested traditional technologies and appropriate policies, and synergized with modern information technology, should promote congruence of enhanced productivity, sustained and healthy ecology, and environment.

Ram Lakhan Singh
Sukanta Mondal

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Chapter 1

Introduction

Ram Lakhan Singh¹ and Sukanta Mondal²

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Chapter Outline

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Critical issues faced by agriculture globally include delivery of human health care, reduction in hunger, and increasing energy supply. The United Nations (UN) predicted that the world population will exceed 9 billion by 2030, thus enhancing the quality and quantity of food production as an inescapable necessity. Global food systems are increasingly threatened by population growth, land degradation, climate change, and other stressors. Due to these factors, agriculture faces numerous challenges, making it harder to accomplish its essential target of feeding the world every year. The challenge is not only to feed more people, but to do so with less available arable land, fewer nonrenewable resources, and less water. The majority of the world's poor people live in rural areas, and agriculture growth might be compelling in lifting rural families out of poverty and hunger. Sustainable agriculture using biotechnology is the need of hour because it contributes more effectively to gains in agricultural productivity, enhancing food security, reduction of poverty and malnutrition, and become more environmentally sustainable. The UN Food and Agriculture Organization (FAO) stated that 70% of this

additional food must come from the utilization of new and existing agricultural technologies. The FAO has also estimated that livestock production would produce almost 20% of worldwide greenhouse gas (GHG) emissions. Despite the environmental challenge, by the end of the next decade, the livestock sector is required to provide 50% of worldwide agricultural output on a value basis. Role of livestock sector is crucial to fulfill growing food demand which is relied upon the increment by 40% by 2030 and shall almost be doubled by 2050.

The completion of the Human Genome Project has provided an array of information about the structure of the genome, which can be utilized to study how the interaction between our genes and factors from the environment like nutrition relates to a state of health or disease. The recent advances in nutrigenomics has widened up the opportunities to contrive our understanding of how nutrients regulate gene expression, protein biosynthesis, and metabolism. Recent innovative biotechnologies such as genetic engineering (GE) and recombinant DNA technology have enormous potential for affecting global food security, human and animal health, environmental health, and overall sustenance of mankind. GE has the potential to provide convincing advantages to transform public health such as improved nourishments, advances for human health, enhanced animal welfare, and reduced environmental impact.

BIOTECHNOLOGY

The Convention on Biological Diversity defined biotechnology as “any technology application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.” Biotechnology deals with the construction of microorganisms, cells, plants, or animals with useful traits by GE, recombinant DNA techniques, tissue culture, embryo transfer, and other methods besides traditional genetic breeding techniques. According to Cartagena Protocol on Biosafety, Modern biotechnology is defined as the manipulation of genetic material and fusion of cells beyond normal breeding barriers with the help of GE in which genes are inserted or deleted through transgenic technologies to create genetically modified organisms (GMOs). It includes modification and enhancement of living organisms at the molecular level using different interdependent components such as genomics, bioinformatics, transformation, molecular breeding, diagnostics, and vaccine technology.

The major steps in recombinant DNA technology are as follows:

- Specific nucleotide sequences (genes) are cut from the DNA of humans, plants, or animals.
- These nucleotide sequences are recombined into circular DNA (plasmid) of certain bacteria. Such DNA is called recombinant DNA.

- The recombinant DNA is reintroduced into bacteria.
- Bacteria divide and a substantial population is produced, each bacterium having the copied plasmid conveying the desired gene.

Modern biotechnology has provided chances to develop more nutritious and better-tasting foods, higher crop yields and plants that are naturally protected from diseases and insects. It allows for the transfer of only one or a few desirable genes, thereby allowing researchers to produce crops with particular beneficial characteristics and diminish undesirable characteristics. Traditional biotechnology such as crosspollination produces numerous, non-selective changes. Modern biotechnology offers effective techniques to tackle food security issues. Biotechnological methods might be utilized to drastically reduce the time necessary to detect foodborne pathogens, toxins, and chemical contaminants, as well as to increase detection sensitivity. Enzymes, antibodies, and microorganisms produced using r-DNA techniques may be used to monitor food production and processing systems for quality control.

SUSTAINABLE AGRICULTURE

As enunciated in the 1990 “Farm Bill,” sustainable agriculture means “an integrated system of plant and animal production practices having a site-specific application that will, over the long term: (1) satisfy human food and fiber needs, (2) enhance environmental quality and the natural resource base upon which the agricultural economy depends, (3) make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls, (4) sustain the economic viability of farm operations, and (5) enhance the quality of life for farmers and society as a whole” (Cohen, Hug, Taddese, & Cook, 1990). Agricultural sustainability is the fruitful administration of assets for agriculture to fulfill changing human needs, while keeping up or enhancing the quality of the environment and conserving natural resources (Gregory, 1989). It includes novel agricultural methods to protect the environment, conserve natural resources, diminish the use of chemical compounds, and promote financial independence. Sustainable agriculture is in fact an economical and environmental necessity in poorer nations where production technologies and frameworks, in which waste constitutes a huge extent of production expenses, cannot be afforded. Thus, advancement should be assessed by a combination of qualitative and quantitative criteria and the effects of agricultural development on social, environmental, and health aspects. Sustainable agricultural framework that is indivisible part of rural development in various nations is actually environmentally flawless, financially and economically reasonable, and socially adequate (Fig. 1.1). Although various factors are included in the sustainability of agricultural framework, supportive institutional and

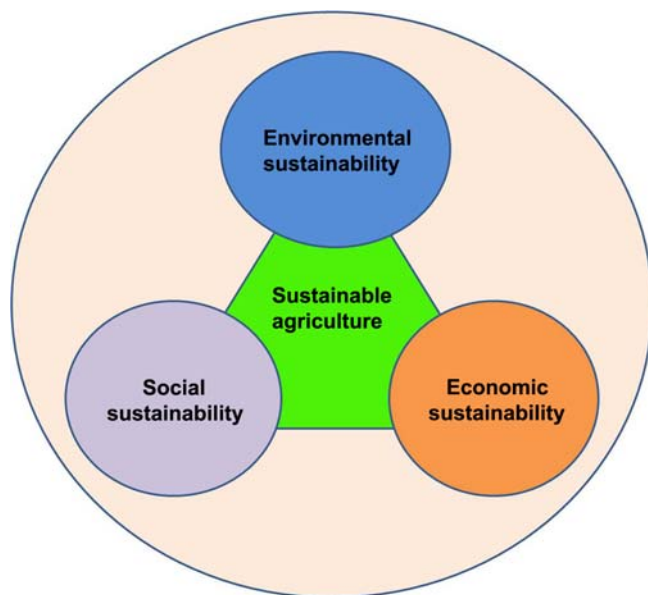


FIGURE 1.1 Components of sustainable agriculture.

infrastructural technologies have an important role in this regard. As such, sustainability and productivity are certain when looking to protect the environment and diminish rural poverty. Sustainable agricultural technologies were broadly acknowledged because of concerns about the environmental impacts of modern agriculture, dependence of agriculture on nonrenewable resources, and long-term productivity of agricultural systems relying on huge external inputs (Leal Filho, 2000). Although many factors are involved in the sustainability of agricultural systems, supportive institutional and infrastructural technologies have a significant role in this respect.

APPLICATION OF BIOTECHNOLOGY FOR SUSTAINABLE AGRICULTURE

Biotechnology has the potential to facilitate and promote sustainable agriculture and rural development. These technologies have environmental benefits, especially considering the fact that renewable genetic inputs are efficient alternatives to dependence on external agrochemical inputs. The potential of genes or genotypes (e.g., varieties or species) to replace renewable resources is highly important in further promotion of sustainable agriculture and rural development. It must be underlined that biotechnology should not be comprehended as a substitute for conventional tools of crop improvement, but incorporating recombinant techniques into traditional breeding programs

could substantially enhance the efficiency of agricultural research and development. The innovations of biotechnology have generally led to the following achievements:

- Better interpretation of plants' functions and reactions to the environment.
- Purposeful objective selection in programs to enhance the efficiency and productivity of crops, trees, farm animals, fish, and quality of food storage.
- Increased crop productivity through increasing resistance to diseases and draught.
- Improved nutritional value: With raised interest in greater nutritional value, taste, and nutritional composition of food products, high-protein GM products with more desirable nutrients, amino acids, and starch levels have been developed for people with inappropriate diets.
- Fresher products: Genetic modification can augment the durability of products. By easier transfer of fresher products, consumers will have more access to complete foods with higher nutritional value. In addition, preventing spoilage, damage, and reduced nutritional value will be facilitated.
- Application of molecular markers (DNA) in making insight and the ability to select main characteristics and limit the variety of possibilities in a farm.
- Molecular tools for elucidation, protection, and application of genetic resources.
- Powerful molecular diagnoses to help detect and manage parasites, pests, and pathogens.
- Making domestic animals and fish resistant to life-threatening diseases.
- Natural benefits: As GE decreases the dependence on insecticides, lower levels of insecticide will remain in food. The leaching of insecticides into groundwater will hence be reduced and farm workers' contact with hazardous and deadly compounds will be minimized.

Genetically Modified Foods

Recent advances in molecular biology and functional genomics demonstrated that related biotechnology products are going to be quite realistic in the near future. Thus, improved crop varieties could be tailored for marginal agroecological regions using GM technology, which have been largely neglected by the green revolution. This technology offers the likelihood of introducing a useful character from firmly related plants without associated detrimental genes or from related species, which do not promptly cross with the crop of interest or from completely unrelated species even in other taxonomic phyla. It opens opportunities for insect/pest/disease control, food fortification with essential vitamins like vitamin A in cereals, micronutrients such as zinc and iron and essential amino acids like lysine, and for producing plants that are

drought tolerant or generally capable of growing well in harsh environments. An important feature of GM technology is its user-friendliness as it is packaged in a convenient form of the seed. The major advantage of using this technology in agriculture is possibility of increase in productivity through use of new varieties that possess properties such as resistance to pest. The damage to the crops is mainly caused by insect larvae and to some extent adult insects. Most of the insects that damage crops belong to Lepidoptera (bollworms), Coleoptera (beetles), Orthoptera (grasshoppers), and Homoptera (aphids). As a result of advances in genetic transformation and gene expression during the last decade, there has been rapid advancement in utilizing GE for crop improvement in terms of herbicide tolerance, pest resistance, and male-sterility systems. The first transgenic plants with *Bacillus thuringiensis* (Bt) genes were produced in 1987. Bt cotton is genetically modified (GM) crop that contains a foreign gene isolated from *B. thuringiensis*. The soil bacterium *B. thuringiensis* produces crystal proteins (Cry proteins) that are toxic to larvae of insects like tobacco budworm, armyworm, beetles, and mosquitoes. The Cry proteins exist as inactive protoxins. When inactive protoxin is ingested by the insect, it is converted into active toxin in the midgut of insects because the alkaline pH of gut solubilizes the crystals. The activated toxin binds to the surface of epithelial cells of midgut and creates pores. This causes swelling and lysis of cells leading to the death of the insect (Larva). A nematode *Meloidogyne incognita* infects tobacco plants and reduces their yield. A novel methodology was adopted to prevent this invasion which was based on the process of RNA interference (RNAi). The specific genes from the parasite are introduced into the plant using *Agrobacterium* as the vector. The genes are introduced in such a way that both sense RNA (from insects) and antisense RNA (from plants) are produced. As these two RNAs are complementary, they form a double-stranded RNA. As result, the parasite cannot line in the transgenic host, and the transgenic plant is protected from the pest. Transgenic plants with insecticidal genes are set to feature prominently in pest management in both developed and the developing nations in future. Such an effort will assume a noteworthy part in minimizing insect-related losses, increase crop production, and enhance the quality of life for the poor rural people. Development and implementation of transgenic plants with insecticidal genes for pest control will lead to (1) reduction in insecticide sprays, (2) increased activity of natural enemies, and (3) integrated pest management (IPM) of secondary pests.

Biotechnology for Sustainable Livestock Production

Superovulation and Embryo Transfer

Manipulation of reproductive processes in domestic animals started in the 1930 with the advent of artificial insemination (AI). Its use became

widespread in the 1960s when AI organizations began to make routine use of frozen semen. Making greater use of the egg cells in the ovaries of the genetically superior animals through superovulation allows greater use of the female. Embryo transfer is a very powerful technology for increasing the productivity in animals (Madan, 2005). Exploitation of female reproductive capacity of valuable donors can be made by superovulating the donors (resulting in more number of ovulations) and subsequent transfer after fertilization into recipients (resulting in more offsprings from donors) even though certain factors are limiting superovulation response in animals. Significant facilitation of import and export for valuable genetic material, development of new breeding concepts, gene conservation by freezing techniques, twin production, introduction of new genes into closely related herds, manipulation of embryos and transgenic animals have also become possible through this technique. The combination of AI and embryo transfer has completely transformed the breeding scheme in bovines. Recent developments in oocyte maturation and in vitro fertilization in farm animals provided a new dimension for improvement of animal reproduction through exploitation of female reproductive capacity.

Somatotrophin in Milk Production

Administration of growth hormone (GH) or bovine somatotrophin (BST) is a potential technological tool for augmenting milk production in dairy animals. BST is a naturally occurring protein hormone produced by anterior pituitary gland and commonly used for enhancing milk production. Recent advances in recombinant DNA technology have made available BST which has similar biological activity to the natural hormone and has been effectively tested in cattle, buffalo, sheep, and goat because of its beneficial effect on metabolism in relation to milk production, growth, and carcass composition.

The impact of BST treatments was found to vary markedly for both short- and long-term treatment. The production response varies from 10% to 25% in cattle and buffaloes with predominant increase in production within 48 hours after treatment. A slight increase in milk fat and decline in protein content have been reported following BST administration which disappeared later on. Somatotrophin has also been found to increase feed intake and feed conversion efficiency by diverting a part of maintenance requirement toward milk production and lowering use of nutrients for body tissue deposition. The milk and meat from BST-treated animals are safe for human consumption because of its degradation by proteolytic enzymes in the digestive tract and biologically inactive in human being.

Gene Transfer and Transgenic

Revolutionary new opportunities for the modification of animal performance have been created by the development of gene transfer technique (Gordon &

Ruddle, 1981; Hammer et al., 1985). Gene transfer offers a powerful approach not only for studying the molecular mechanisms of animal reproduction, animal growth and development but also for developing manipulating techniques of animal growth and reproductive efficiency. The first production of transgenic mice excited the animal scientists with hopes of doing likewise in domestic livestock with the possibilities for targeting gene expression exclusively to skeletal muscle for altering the meat product or to the mammary gland for altering composition of milk to include different proteins of pharmaceutical importance. Insertion of foreign DNA sequences into mammalian germ line is done by microinjection of foreign DNA into undivided fertilized egg using retrovirus. The potential areas for gene transfer are the production of huge amounts of specific proteins (blood factor VIII and IX, human serum albumin, etc.), change or improvement in composition of milk, introduction of disease-resistant gene for economically important infectious disease, introduction of GH gene for gross manipulation of animal phenotypes, and introduction of gene-encoding metabolic enzymes. The transfer of GH gene has been performed in many species of animals but has shown much more success in fish and pigs than in other farm animals such as cattle, sheep, goat, and poultry. However, GH transgenic pigs had several health and reproductive problems because of unregulatable GH expression.

Gene transfer seems more difficult and complicated in farm animals than in mice. The efficiency and production of transgenic animal is very low and varies with animal species. Because of low efficiency, production of transgenic farm animals requires large intellectual, technical and financial investment, huge animal resources, and more time expenditure. Therefore, the commercial applications of transgenic farm animals for improving efficiency of reproduction and growth won't be possible until the efficiency of transgenic techniques is dramatically enhanced.

Vaccines and Diagnostics

Biotechnology has revolutionized research on the development of vaccines resulting in new insights into the molecular mechanisms of pathogenesis and the immunology of infectious diseases and has opened up perspectives of modern vaccines such as recombinant DNA vaccines, and chemically synthesized peptide vaccines. Vaccines produced by conventional means are live and killed microorganisms. Live vaccines contain a replicating agent, whereas killed vaccine contains a noninfectious agent and an adjuvant. In general, live vaccines stimulate the strongest protective immunity but may present hazards like contamination with adventitious agents, whereas killed vaccines are relatively poor immunogen but are usually safer. Only a few genetically engineered vaccines are on the market such as recombinant DNA vaccines against *Escherichia coli* and *hepatitis B vaccine*. At present, enormous quantum of research is going on in this area in an effort to develop

new, safer, and more efficacious vaccines which give the highest protection to the animal economically. Whereas conventional vaccine was, to a certain degree, developed by trial and error procedure, biotechnology offers new strategies for well-defined engineering of vaccines. However, before vaccines can be designed, more knowledge is required on the nature of microbial immunogens, the mechanism underlying the microbes, the molecular pathogenesis of an infection, and the immunological processes responsible for induction of protective immunity.

Before live recombinant DNA vaccines are registered, extensive safety assessment studies should be conducted. More information on efficiency and safety testing with *Vaccinia*-vectored vaccines are required before these vaccines can be approved for use in animals. *Vaccinia* virus has the potential for use as a vector mainly in developing vaccines for severe infectious diseases such as AIDS, malaria, and leprosy. There are still a large number of animal diseases for which protective vaccines are not yet available, especially against parasitic diseases. Some antigens have been virtually impossible to incorporate into vaccines because of difficulty in growing the microorganisms or isolating the antigens or because of complexity or inability to adopt them to a viable production method.

Gene Knockout

Gene knockout or gene disruption is a molecular approach that specifically silences a target gene. There are many genes that are involved in regulation of growth and nutrient deposition in livestock species. Some genes have a general or local (tissue specific) growth including function that causes retarded growth of whole body or specific tissue. Knockout of these genes could lead to overgrowth. Type II Insulin like growth factor receptor gene (IGF 2R) may be an example of genes that have a general growth inhibiting function. Knockout mice lacking IGF 2R showed the increase of IGF-2 levels. Prenatal growth was improved with birth weight being 1.4 times higher than that of wild-type control. Myostatin is an example of gene having local growth inhibiting effect. It is preferentially expressed in adult skeletal muscle. Muscle mass is enhanced by gene knockout of myostatin gene. Commercial application of this technique won't be feasible until all the biological impacts of myostatin knockout in farm animals are evaluated.

Gene Therapy

Gene therapy is another approach of introducing exogenous gene into animals. Gene therapy is originally developed as a mean to correct genetically based diseases in humans. However, this technique may also be used to deliver genes that can alter growth and body compositions. Very limited information is available about its use in growth manipulation. In laboratory animals, when GM myoblast expressing human GH were delivered

systematically, circulating GH concentration was increased. If this approach succeeds in domestic animals, repeated injection of recombinant GH would be avoided. Another potential application of gene therapy for growth control may be the direct injection of plasmid DNA-encoding growth-promoting genes into muscle tissue. Skeletal muscle has become most attractive site for expression of foreign genes. It is promising to use skeletal muscle as an artificial endocrine tissue to produce a variety of physiologically active proteins for therapeutic and production purposes.

Biotechnologies for Gut Microorganism

Biotechnology can be used for improving metabolism and activity of gut microorganisms that are very important for animal health and growth. This can be done by three approaches which are as follows:

- Application of biotechnological products to improve the gut ecosystem and promote the growth of beneficial bacteria, for example, pre- and probiotics.
- Genetic modification of microorganisms naturally present in the gut to enhance their capacity of defined functions or to add new functions. Introductions of diverse genes into gut microorganisms have been investigated. The GM microorganisms are able either to digest fibrous component and lignin of forages, to degrade toxin, to synthesize essential amino acids, to reduce methane formation, or to tolerate acids.
- Introduction of new species or strains of microorganisms into the gut.

Biotechnology for gut microorganism is far from being commercially applicable because of technical problems and public concerns.

Use of Microbes for Sustainable Agriculture

Microorganisms play vital role in the functioning of ecosystems and in maintaining a sustainable agriculture. Sustainable agriculture is the need of present world as it offers the potential to meet our agricultural necessities. The plant-associated microbes show great diversity of living habits whose saprophytic or symbiotic relationships with the plant could be beneficial. Most of these microbes reside in the rhizospheric region, but some of them, designated as endophytes, successfully penetrate and live inside plant tissues. Several soil microbial communities are exploited for the purpose of sustainable and healthy crop production, while preserving the biosphere. These soil microorganisms play important roles in agriculture primarily by improving plant nutrition, soil quality and health (Lugtenberg, 2015). The advantages of applying microbes in agricultural practices include not only sustainable agriculture but also other ecosystem-associated benefits. These include ecosystem restoration, enhancing resilience of plant communities, recovering of

endangered flora, adaptive strategies for diversity conservation, etc. (Barea et al., 2013). For that reason, several strategies are proposed for a more effective utilization of beneficial microbial population to help sustainable environment-friendly agrotechnological practices. The major aim of using microbes is required for continuous supply of essential nutrient for growth and plant protection. As the interactions between microbial population and plants are influenced by several biotic and abiotic factors and agronomic managements, the effect of environmental stress factors must be overcome, mainly in the present scenario of global climate change, as they severely damage the plant–microbe interactions.

Impact of Climate Change on Agriculture and Livestock Production

Agriculture

Agriculture is essential for food security in two ways: It produces the food people eat, and (perhaps considerably more important) it gives the primary source of sustenance for 36% of the world's total workforce. If agricultural production in the low-income developing countries of Asia and Africa is unfavorably influenced by climate change, the sustenance of large numbers of the poor people belonging to rural areas will be put at risk and their vulnerability to food insecurity increased. The major effect on crop is due to shortening of crop duration which is related to the thermal environment. Increase in temperature will hasten crop maturity. In annual crops, the shortening of crop duration may vary from 2 to 3 weeks, thus adversely impacting productivity. Extremely high and low temperatures cause physical injuries to crop plants and harm the grain. Injuries are dispensed by high temperatures on the exposed area of plants, scorching leaves and dehydrating the plant. Young seedlings additionally get dehydrated rapidly when soil temperature increases. The rise in temperature in lower latitude regions accelerates the rate of respiration, excessively leading to suboptimal growth. Rainfed rice yields in India are expected to reduce by ~6% in the 2020 scenario, whereas under the 2050 and 2080 scenarios, the yields are projected to decrease only marginally (<2.5%) (Soora et al., 2013). Early modeling studies on wheat indicated that with every rise of 1°C in mean temperature, India could lose 4–5 million tonnes of wheat (Aggarwal, 2008). However, using adaptation strategies such as changing the planting dates and utilizing different varieties, it is possible to reduce the losses. By adapting certain agronomic procedures, it was assessed that at a 1°C rise, 3 million tonnes could be restored. Winter (Rabi) maize grain yield in India is projected to reduce with increase in temperature in Mid Indo-Gangetic Plains (MIGP), and Southern Plateau (SP). Spatio-temporal variations in projected changes in temperature and rainfall are likely to lead to differential impacts in different regions. In particular,

monsoon season yield can reduce mostly in SP (up to 35%), and winter yield will reduce in MIGP (up to 55%), whereas upper IGP yields will be relatively unaffected. Climate change may increase production of potatoes in Punjab, Haryana, and western and central Uttar Pradesh by 3% to 7% in A1B 2030 scenario, but in the rest of India, particularly West Bengal and SP region, the production may decline by 4%–16%. It is primarily attributed to the rise in mean minimum temperature during tuber development stages which affects potato yield. The increase in temperature due to climate change may decrease harvest index (HI) of this crop grown in large parts of Maharashtra, parts of Karnataka and Andhra Pradesh. Even though, in the traditional potato-growing belt in the IGP, the HI may remain more or less stable but pockets of high HI are likely to diminish (Soora, Singh, Agarwal, Rao, & Venkateswarlu, 2012).

The level of GHGs [carbon dioxide (CO_2), water vapor (H_2O), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF_6)] is increased due to anthropogenic activities. The increased level of GHGs contribute to an overall increase of the earth's temperature, leading to global warming. The average global surface temperature has increased by 0.74°C since the late 19th century and is expected to increase by 1.4°C to 5.8°C by 2100 AD with remarkable regional variations (IPCC, 2007). The elevated CO_2 levels can influence the crop yields. Higher CO_2 level has been associated with diminished protein and nitrogen content in alfalfa and soybean plants, bringing about loss of quality. Diminished grain and forage quality can decrease the capacity of pasture and range land to support grazing livestock. Though rising CO_2 can stimulate plant growth, it also reduces the nutritional value of most food crops. Increased levels of atmospheric carbon dioxide decrease the concentrations of protein and essential minerals in most plant species, including wheat, soybeans, and rice. This immediate impact of increasing CO_2 level on the nutritional value of crops represents a potential danger to human health. Human health is also threatened by increased pesticide use due to increased pest pressures and reductions in the efficacy of pesticides (Ahmad, Arif, Ahmad, & Attique, 1998).

Livestock Production

Climate change has adverse impact on animals influencing distribution, growth, incidence of diseases, availability of prey, productivity and in extreme cases, extinction of species because of habitat loss (Nardone, Ronchi, Lacetera, Raniere, & Bernabucci, 2010). Although both domestic and wild animals ranging from insects, amphibians, birds to mammals have been accounted for to be influenced by global climate change, information on direct impact of climate change on animals is scarce (Nardone et al., 2010; Padodara & Ninan, 2013). The major impacts of climate change are

owing to increased surrounding temperature. Climate change has complex impacts on domestic animal production system influencing feed supply, challenging thermoregulatory mechanism resulting thermal stress, emerging new diseases because of change in epidemiology of diseases and bringing about other indirect effects. Thermal stress is one of the major climatic challenges confronted by the domestic animals (West, 2003). The global warming may further provoke the situation and even incite new scenes of thermal stress condition (Padodara & Ninan, 2013). Global warming has two-way effects on animal production system. In one hand, it directly influences the health, reproduction, nutrition of the animals bringing about poor performance, inferior product quality, outbreak of novel diseases, whereas on the other hand, there are indirect consequences for animal production due to change in soil fertility, reduction in preferred vegetation, degradation of range land, desertification, and decrease in feed stuffs production (Nardone et al., 2010; Padodara & Ninan, 2013). Heat stress has been found to reduce daily milk yield by 21% in Tunisia, Florida (Mallonee, Beede, Collier, & Wilcox, 1985), and in South Africa (Du Preez, Giesecke, & Hattingh, 1990). A 10%–14% reduction in milk production was observed in Argentina when dairy cows were subjected to heat wave conditions associated with climate change. The midlactating dairy cows were the most heat sensitive contrasted with their early and late lactating counterparts.

Heat stress affects the fertility of livestock species during summer months. The interruption of reproduction during heat stress is brought about by the failure of the animal to adapt with heat stress, leading to a rise in body temperature above its regulated set point, which can compromise the functioning of the germ cells and the viability of an early developing embryo (Hansen, 2009). Although nuclear maturation seems to be minimally impacted by heat stress, several lines of evidence indicate that elevated temperature impacts certain aspects of oocyte cytoplasmic maturation. The effect of heat stress on fertility is dependent upon duration and severity of the stress imposed. Exposure of superovulated heifers to 42°C and 75% relative humidity for 10 hours at the onset of estrus has been found to raise respiration rates by more than 200% and rectal temperatures from 38.9°C to 41.3°C (Putney, Mullins, Thatcher, Drost, & Gross, 1989). Exposure of sheep to high temperature and humidity (50°C to 52.7°C and 60% to 65% relative humidity) at the time of breeding increased the proportion of abnormal oocytes and prevented lambing (Dutt, 1963). Exposure of oocytes to elevated temperature during in vitro maturation brought about reduced embryo development suggesting that some of the negative impacts of heat stress are due to a direct impact on the maturing oocyte. The timing of exposure to elevated temperature during oocyte maturation is important: Effects of culture at 41°C for 12 hours on blastocyst development after fertilization were more pronounced during the first half of maturation than the last 12 hours (Edwards & Hansen, 1996).

The embryonic developmental rate and hatched blastocyst rate are reduced when embryos were exposed to high temperature *in vitro*. Heat shock caused a greater reduction in the proportion of cultured two-cell embryos that developed to the blastocyst stage compared to heat shock of four- to eight-cell embryos, whereas morulae were unaffected by heat shock. Early embryos (<8- to 16-cell stage) would be more susceptible to heat shock as these embryos are transcriptionally quiescent and unable to produce defensive molecules such as heat shock protein 70 (HSP70) in response to heat shock. Hyperthermic conditions influence the viability and developmental abilities of oocytes and embryos relying upon both the temperature and the duration of exposure. The use of supraphysiological temperatures induces more severe consequences as exposure of murine oocytes to 41°C (4°C above normal) for 17 hours completely inhibited meiotic maturation, whereas temperatures of 40°C or higher reduced normal chromosome spreads and increased abnormal ploidy (Fiorenza & Mangia, 1992). Direct heat stress of murine oocytes at 40°C during maturation resulted in abnormal chromosome morphology and number (Fiorenza & Mangia, 1992). Absence of polar bodies in matured bovine oocytes after heat stress suggested possible inhibition of meiosis. When heat-stressed bovine ova (41.0°C, 7 first 12 hours of maturation) were fertilized earlier, there was an increase in the number of embryos that developed into blastocysts, with the best development occurring when IVF was performed 18 hours after the onset of maturation.

Nanotechnology for Sustainable Agriculture

In the present century, nanotechnology has emerged with great influence on public lives, global economy, and industries. It has the potential to transform agricultural industry with novel tools for the rapid disease diagnosis, molecular treatment of diseases, improving absorption capacity of nutrients by plants, etc. In addition, the uses of nanomaterials particularly for agricultural exercise are required for increase in yield through nutrient optimization, improving the fertilization process and minimizing the requirements of plant protection products (Huang, Wang, Liu, Hou, & Li, 2015). Nanotechnology products such as nanomaterials, nanofibers, nanostructures, and nanotubes with unique physical and chemical properties play important role in plants and animal breeding. Nanomaterials can also be utilized for analysis of soil samples, delivery of nutrients and pesticides in the plants (Srilatha, 2011), and waste water treatment. The targeted delivery of nanomaterials not only reduces the harm to nontarget plant tissues but also decreases the quantity of harmful chemicals that spoil the environment. Hence, nanotechnology will also protect the biosphere indirectly through the use of alternative energy supplies and is considered environment-friendly technique. In agricultural sector, nanotechnology is used to expand the GM crops, animal production inputs, and precision farming techniques.

Biosafety Aspects

The role of biotechnology in agriculture includes a range of tools that are employed to understand and modify the genetic make-up of organisms for use in the generation or processing of agricultural products. Biotechnology is being utilized to address issues in all aspects of agricultural production and processing. This includes genomics, proteomics, transformation, molecular breeding, diagnostics, and vaccine technology (FAO, 2004). Modern biotechnology, including genetic modification and production of GMOs, could possibly give powerful tools for the sustainable development of agriculture and in addition convey food security for growing population around the world. However, with increasing applications of modern biotechnology, there is a vital need to ensure that these tools are utilized sensibly. It is, therefore, important that the development or utilization of the products of modern biotechnology is prior assessed for potential risks to human health and safety, and the environment. To ensure appropriate utilization of this technology, biosafety rules, regulations, legislations, risk assessment, risk management, and monitoring mechanisms have been developed in different countries around the world. The biosafety assessment should be done from the social, ethical, economic, health, and environmental perspective.

Biosafety refers to the policies and methodology adapted to fortify the safe and ecologically sustainable usage of biotechnology that results to develop GMOs. Various international regulatory agencies such as International Plant Protection Convention (IPPC), International Epizootics Organisation (OIE), Codex Alimentarius (Codex), Food and Agricultural Organisation (FAO), World Health Organisation (WHO), World Trade Organisation (WTO), and Organisation of Economic Co-operation and Development (OECD) are there to regulate and govern different aspects of food safety and agricultural biotechnology. These organizations are responsible to set up standards for health, safety, and labeling for GM foods, develop testing systems to guarantee the standards are met, provide rules for permissible policies, and make frameworks to manage disputes (IFPRI, 2003).

Genetic Engineering and Public Perception

GE is a process which involves the genetic modification or manipulation of genes. This technique allows novel traits from various organisms to be introduced into crops, livestock, and microorganisms thus breaking the species barrier (Asaye, Biyazen, & Girma, 2014). GE technology offers chances to supplement traditional techniques and regarded as a promising way to ensure the sustainable agricultural productivity. Important factors influencing the public attitudes are the perception of benefits and risks, and knowledge of GMOs (Lucht, 2015). GE in crop and livestock production has a growing number of viable advantages, such as production of herbicide and insect-

TABLE 1.1 Probable Benefits and Risk Posed by Genetically Modified Organisms

Benefits	Risks
● Improved and increased agricultural productivity ● Increased food production with improved nutritional value ● Herbicide-, insecticide-resistant crops ● Disease-resistant crops and livestock ● Reduced environmental footprint of agriculture ● Improved human health by production of novel therapeutic proteins, drugs, vaccines	Ecological and environmental issues ● Impacts on nontarget species ● Resistance to insecticides ● Threat to biodiversity and to genetic diversity Health-related issues ● Allergens and toxins ● Antibiotic stability Religious, cultural, and ethical Issues

resistant transgenic crops, production of transgenic livestock resistant to diseases, increasing and improved productivity of crops and livestock as well as their products, and production of therapeutics. The major GM crops are soybean, cotton, maize, and oilseed rape, and the most common genetically introduced traits are herbicide (glyphosate) tolerance and Bt toxins. However, these benefits have not been universal. There are many controversies about the risks posed by GMOs (Table 1.1) as under.

- New genes could modify the expression of native genes and thus may have unexpected secondary effects.
- Transgenic crops modified to be resistant to a specific pest or disease may negatively affect nontarget species that are harmless or beneficial.
- Insects can develop resistance to Bt (*B. thuringiensis*) toxins, or weeds develop resistance to glyphosate, reducing the effectiveness of their control method.
- Allergenic and immune system reactions may be due to the new substances contained in GMOs.
- Antibiotic-resistant marker genes used in GE might be transferred to pathogens in the gut of animals or humans resulting in antibiotic resistance. Disease triggered by these pathogens could no longer be treated with these antibiotics because pathogen may be resistant to these antibiotics.

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Chapter 2

Biotechnological Tools to Enhance Sustainable Production

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INTRODUCTION

Since the beginning of the 20th century various tools have been introduced to extend the potential for breeding new plant varieties. Cell biology, plant tissue culture, micropropagation, embryo rescue, and protoplast fusion techniques allow the production of various uniform plants and the crossing of distant related crop plants. Genetic transformation through genetic engineering utilized recombinant deoxyribonucleic-acid (DNA) technology to enlarge the gene pool available to plant breeders. Several recent techniques permit

for site-directed mutagenesis (SDM) of plant genes (to knock out or modify gene functions) and the targeted deletion or insertion of genes into plants genetic background. Plant transformation has become widely adopted as a method both to understand plant works and to improve crop plants characteristics. The biotechnological tools have been used to improve the ability to detect, treat, and prevent several diseases of plants and livestock. Plant transformation depends on the stable integration of foreign gene into the genome of plant. Various methods have been developed to achieve this, and many plant species have been transformed successfully. The first crops produced by genetic transformation technologies are herbicide tolerant and pest resistant varieties, commercially available for cultivation in the mid-1990s. The biotechnological tools have important role in crop improvement in terms of increased yields, herbicide, and drought tolerant crop varieties, insect and pest-resistant crop varieties (Adenle, Sowe, Parayil, & Aginam, 2012). In recent years, biotechnological tools have been successfully utilized as powerful means to develop various livestock products such as milk and meat products. These tools can also be applied in production of high yielding animal, disease-resistant livestock, improvement of animal origin products quality, functional and designer livestock products, enzymes, biopreservation of livestock products, production of hormones, efficient by product utilization, and quality control (Gupta & Savalia, 2012).

NEED FOR BIOTECHNOLOGICAL TOOLS FOR SUSTAINABLE AGRICULTURAL PRODUCTION

Humans developed agriculture approximately 10,000 years back when they started to harvest and cultivate specific plants to produce food for their sustenance. After that, agriculture began independently in various parts of the globe for the cultivation of specific crops and domestication of animals. Early farmers selected the plants and seeds on the basis of faster growth, higher yields, pest and disease resistance, larger seeds, or sweeter fruits (Hallauer, 2011). Plant breeding appeared when man discovered that crop plants could be artificially mated or crosspollinated to have the capacity to improve the characters of the plant. With the development in the field of plant breeding, breeders understood better how to select superior plants and breed them to develop new and improved varieties of different crops (Lee, 2014). The traditional plant breeding methods have been used to develop new varieties of crops for hundreds of years. However, these methods can no longer sustain the global demand with the increasing population, reduction in agricultural resources such as land and water, and the apparent plateauing of the yield curve of the staple crops. According to the Food and Agriculture Organization (FAO), more than 800 million people in the world do not have access to sufficient food to meet their basic needs (FAO, 1996). This is one of the most crucial issues in the 21st century. Thus, new technologies for the improvement of agricultural production should be developed and utilized.

Implementation of effective and successful agricultural development strategies is needed for sustainable food production and to ensure food security.

Recent advances of modern biotechnology tools in agricultural applications contribute to sustainable gains in agricultural productivity, reducing poverty, and enhancing food security. The improvement in agricultural production using biotechnological tools contributes significantly to fulfill the desired nutritional requirements of increasing population of the world (Sharma, Dwivedi, & Jha, 2010). Biotechnology tools hold great promise for increasing the world's food supply and improving the quality of food. These tools can be used for improvement of crop and livestock production in order to meet the demands of the consumer within economic, environmental, and ethical constraints imposed by society.

Biotechnology tool consists of a gradient of technologies, ranging from traditional techniques to new innovative techniques, such as genetic engineering and genomics which play a major role in agricultural sustainability. Using these tools, genetic resources can be more precisely characterized, efficiently improved and tailored to specific needs. These tools contribute to crops and animal production by improving the environmental component of the production systems as well as by improving the genetic make-up of crops and livestock. Biotechnological tools are widely used to increase not only the number of a species of crop varieties and livestock to meet the requirement for world demand of agricultural products but also for endangered species to enhance the propagation and sustaining the current levels of biodiversity and genetic diversity (Abdullah, Khadijah, Embong, & Soh, 2011). The new genetic engineering or recombinant DNA techniques allow the specific identification, isolation, and alteration of genes and their reintroduction into living organisms to produce transgenic varieties of crops and animals. These new techniques are supplementing and extending traditional breeding methods to enhance the production of food, fiber, and other agricultural products.

BIOTECHNOLOGICAL TOOLS FOR CROP IMPROVEMENT

Plant Breeding and Marker-Assisted Selection

Plant breeding is a method for the creation, selection, and fixation of superior plant phenotypes in the development of improved crop varieties which fulfills the need of farmers and consumers. Primary goals of plant breeding with agricultural crops have aim to improve yields, nutritional qualities, and other traits of profitable value. Globally, the plant breeding model has been very much successful, with the development of hybrid maize (*Zea mays*), the introduction of wheat (*Triticum aestivum*), and rice (*Oryza sativa*) varieties that initiate the Green Revolution (Everson & Golin, 2003). Further advances in our understanding of plant biology, quantitative genetics, cytogenetics, biotechnology, molecular biology, and, most recently, genomics led to increase the scientific base and its application in plant breeding process

(Varshney, Hoisington, & Tyagi, 2006). The process of developing new crop varieties through breeding tool involves several steps and can take 10 to 25 years depending on the individual crop. Furthermore, applications of agricultural biotechnology have significantly shortened the time; it takes up to 7–10 years for new crop varieties to be developed. Marker-assisted selection (MAS) is a tool for easier and faster selection of beneficial plant traits. DNA is the source of different traits and physical features of plants. The DNA occurs in pairs of chromosomes (strands of genetic material), one coming from each parent. The genes are specific segments of each chromosome which specifies the plant's characteristics. All of the genes together make up whole plant genome. Some traits are controlled by only one gene (e.g., flower color), whereas the other may be controlled by many genes (e.g., crop yield or starch content). Traditionally, plant breeders have selected plants based on their visible or measurable traits, called the phenotype. But this selection procedure can be slow, difficult, influenced by the environment and costly not only in the development itself but also for the economy, as farmers suffer crop losses. As an alternative, plant breeders now exploit molecular MAS. Molecular marker is gene or DNA sequence with a known location on chromosome which are used to identify specific genes. The markers are positioned near the DNA sequence of the desired specific gene. Because the markers and the genes are close in position on the same chromosome, they tend to stay together as each generation of plant is produced. This is called genetic linkage which helps scientist to predict whether a plant will have the desired gene or not. Previously, scientists formed very simple genetic maps using conventional techniques. It was observed long ago that as lineages of plants were crossed, some traits consistently appeared together in the new generations because of genetic linkage. Due to linkage phenomenon scientists concentrated on only a few traits in each attempt of cross breeding, so it requires many crosses to prepare even for a very simple genetic map. Molecular marker-assisted breeding technique is utilized for efficient introgression of important genes into various crops such as increased beta carotene content, bacterial blight resistance, and submergence tolerance in rice. However, conventional plant breeding can no longer fulfill the global demand due to increasing demographic structure, decline in agricultural resources such as land and water. Therefore, new crop improvement technologies were developed and used, like mutation breeding. Some traits arise spontaneously through a process called mutation, but the natural rate of mutation is very slow and unreliable to produce desired plants. In the late 1920s, researchers succeeded to generate large number of these variations or mutations by exposing plants to X-rays and other mutagens. Plants were exposed to protons, neutrons, gamma rays, alpha particles, and beta particles to see whether they induce useful mutations or not. Sodium azide and ethyl methanesulfonate are the chemicals which were successfully used to generate mutations.

Plant Tissue Culture and Micropropagation

Plants typically reproduce through sexual means that they have flowers and seeds to produce the next generation. Egg cells in the flowers are fertilized by pollen grains from the stamens of the flower of the same plant (self-pollination) or another plant (cross pollination). Each of these sexual cells contains genetic material in the form of DNA. But in some plants, sexual reproduction is not a common mode of producing next generation. In light of this, plant scientists have developed tissue culture technique to help plant breeders in this task. The technique of plant tissue culture is employed for growing single plant cells, tissues, and organs under in vitro conditions to regenerate and propagate whole plants. Tissue culture is commonly used as a broad term to explain all types of plant cultures for example callus, anther, meristem, cell, root, shoot, protoplast, endosperm, ovary, embryo, and organ cultures. It relies on the phenomenon of cell totipotency, potential of single cells to divide, to produce all the differentiated cells of particular organs and to regenerate into an entire plant. Tissue culture is the cultivation of plant cells, tissues, or organs on specially designed nutrient media. Under the suitable culture conditions, whole plant can be formed from a single cell. Plant tissue culture have great impact on both agriculture and industry and have significant role in the development of agricultural sciences in recent times, and today, it is regarded as an essential tool in modern agriculture (Garcia-Gonzales, Quiroz, Carrasco, & Caligari, 2010). Tissue culture permits the production of genetically homogeneous, disease-free plant material (Chatenet et al., 2001) and useful tool for the induction of somaclonal variation (Marino & Battistini, 1990). In vitro cultures of mature and/or immature zygotic embryos are used to recover plants obtained from intergeneric and interspecific crosses that do not produce fertile or viable seeds. In addition, genetic engineering technique can also be used to improve number of crop varieties with high yield potential and resistance against pests, herbicides, diseases, etc. Genetic-transformation technology relies on the technical aspects of plant tissue culture and molecular biology for production of improved crop varieties, production of secondary metabolites, production of virus free plants, and production of crop varieties which are resistant to drought, salinity, and heat stress.

Micropropagation or clonal propagation has become an important part for in vitro vegetative propagation of plants by tissue culture to produce genetically similar copies of many plants. Several techniques for in vitro plant propagation have been developed, for example, culture of apical meristems, induction of axillary and adventitious shoots, and plant regeneration by somatic embryogenesis and/or organogenesis (Gautheret, 1985). Sexually propagated plant display a large amount of heterogeneity, whereas asexual reproduction (by multiplication of vegetative parts) gives rise to genetically identical copies of parent plant. Micropropagation proves useful for propagation of sexually sterile species (e.g., triploids, aneuploids which can't be continued by seeds), seedless plants (e.g., banana), cross-bred perennials where

heterozygosity is to be preserved, mutant lines such as auxotrophs which can't be propagated in vivo and disease free planting material of ornamentals and fruit trees. Micropropagation offers some advantages over conventional propagation methods which are described as follows:

1. Genotype constitution is preserved as there is lesser variation in somatic embryo
2. Easier transport and storage is offered by small-size propagules and their capacity to grow in soil less medium
3. In vitro growing conditions are totally under control for the production of planting material
4. Reduced growth cycle and rapid multiplication of shoot and roots
5. Through meristem culture, virus free plants can be raised and maintained

Genetic Engineering and Genetically Modified Crops

The growing demand for food poses major challenges for growing population throughout the world. The term “genetically modified” (GM) refers to the transfer of foreign genes into host organisms by using a series of techniques for cloning, splicing DNA segments together, and inserting genes into cells (Fig. 2.1). Together, these techniques are known as recombinant DNA

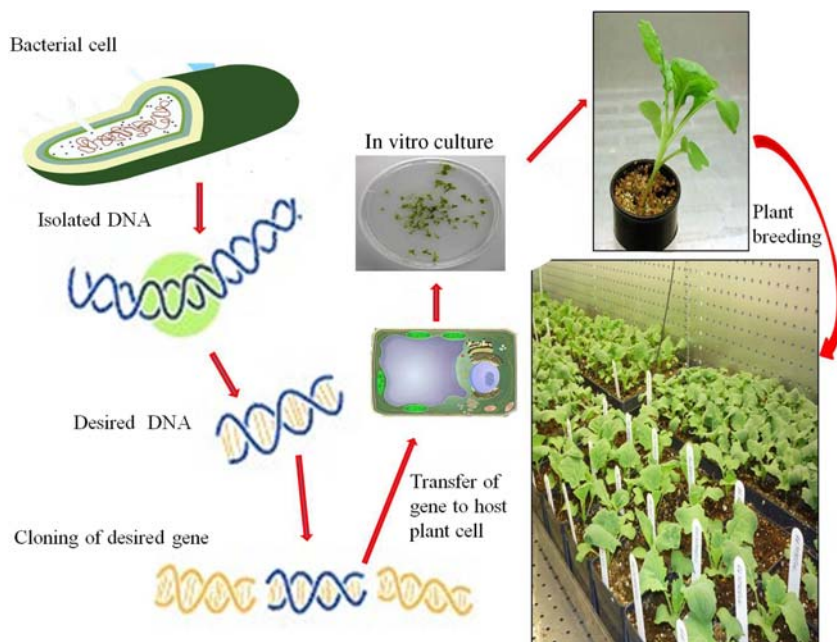


FIGURE 2.1 Method of production of GM plants.

technology (RDT). Other terms used for GM crops derived from them are GM organism (GMO), genetically engineered (GE), bioengineered, and transgenic. A GM crop plant is one which has at least one transgene from another crop plant of the same species or from an entirely different species, inserted artificially for some beneficial purposes (Bryant, 2001). Most of the GM plants are developed to confer resistance against herbicide, insect, fungal, and viral pathogen to cope up the loss of crop yield. Other beneficial traits may also be developed such as salt or drought tolerance and improved storage, quality characteristics. On a global scale, farmers have rapidly attracted toward GM crops. In the year 1992, first commercial GM crop was sown in China. The United States, Argentina, Brazil, Canada, and India are the countries with the largest area of GM crops today (GM Compass, 2009), with a total area of 160 million hectare in 2011 (James, 2011). Most GM crops are produced by using one of the two strategies or approaches; biolistic method or *Agrobacterium-tumefaciens*-mediated transformation technology. Both methods target plant cells that are growing in vitro. Scientists can generate plant tissue cultures from various types of plant tissues, and these cultured cells will grow in either liquid cultures or on the surface of solid growth media. The biolistic method is a physical method of introducing DNA into cells. Particles of heavy metals such as gold or tungsten are coated with the DNA that will transform the cells. These particles are fired at high speed into plant cells in vitro by using a device called a gene gun. Although biolistic methods are successful for a wide range of plant species, but *Agrobacterium*-mediated transformation achieves improved transformation rate. *Agrobacterium tumefaciens* (also called *Rhizobium radiobacter*) is a soil-borne, rod-shaped bacteria that can infect plant cells and cause crown gall disease. These characteristics are conferred by a 200-kb tumor-inducing plasmid called a Ti plasmid. After infection with *Agrobacterium*, the Ti plasmid integrates a segment of its 20-kb DNA known as transfer DNA (T-DNA) into random locations within the plant genome. Some examples of GM crops are described in the following sections.

Herbicide Tolerance

Worldwide, about 10% of plant crop are damaged by weeds. Weeds have significant effect on the yield and crop quality as a result of competition for light and nutrients. Farmer uses weed killers (herbicides) to control wide range of weeds. This herbicide not only kills the weeds, but they also damage the crop plants. Herbicide tolerance was the first GM trait tested on field for commercial production. The majority of these varieties have a bacterial origin gene that confers resistance to the broad spectrum herbicide glyphosate. In the USA, where the large-scale GM crops are grown, the proportion of herbicide tolerant cotton rose from 10% in 1997 to 56% in 2001.

Pest Resistance

This is the second-most important class of GM crop that makes plant resistant to agricultural pests. Insect-mediated loss is one of the most common threats to worldwide crop production. Farmers kill insect pests using predatory organisms and crop rotation, as well as using insecticides. The most widely used GM insect resistant crops are the Bt crops which are produced by transfer of toxin coding gene from bacteria *Bacillus thuringiensis*.

Disease Resistance

There are many species of fungi, bacteria, viruses, and protozoans that cause plant diseases. It was estimated that crop loss in the United States alone cost some US\$33 billion per annum due to pathogen related diseases. Plant pathologists are working to create disease-resistant plants with genetic engineering and some crops are released which are disease resistant.

Molecular Pharming

Molecular pharming is described as an experimental application of biotechnology to GM crops for the production of proteins and chemicals for pharmaceutical and other commercial uses (Franken, Teuschel, & Hain, 1997). It utilizes heterologous protein expression systems for example plants, for the large-scale production of recombinant proteins that are therapeutically important (Table 2.1). Its purpose is to provide a safe and low-cost method for the mass production of recombinant pharmaceutical proteins. Plant molecular pharming is the process of growing plants in agriculture to produce pharmaceutical or other industrially important compounds instead of food, feed, or fiber. The use of plants and plant extracts for medicinal value thrived until the 17th century when more scientific pharmacological treatments were recommended (Trevelyan, 1993). It was estimated that one fourth of the currently used medicines still have a plant origin (Winslow & Kroll, 1998). Tools of genetic engineering have recently unwrapped new prospects for using plants as production factories for biopharmaceuticals. First pharmaceutically important protein that was expressed in transgenic tobacco was human growth hormone. After that, transgenic plants expressing therapeutics, vaccines, industrial enzymes, antibodies, nutraceuticals, and other pharmaceutical proteins have been produced (Ma, Drake, Chargelegue, Obregon, & Prada, 2005). Although both prokaryotic and eukaryotic systems have been utilized to express recombinant proteins, but prokaryotic production systems are comparatively inexpensive and convenient as compared to mammalian systems regarding requirement of technology/equipment. Plants are now the production factory of pharmaceutically important proteins such as mammalian antibodies blood substitutes and vaccines. Plants produce a large amount of biomass and protein production can be enhanced by using plant suspension

TABLE 2.1 Important Pharmaceutical Proteins Produced by Plants

Protein	Host Plant	Application/Comments
α -Interferon	Rice, turnip	First human pharmaceutical protein produced in rice
Collagen	Tobacco	First structural human protein polymer produced in plants
Epidermal growth factor	Tobacco	Wound repair and control of cell proliferation
Erythropoietin	Tobacco	First human protein produced in tobacco suspension cells
Growth hormone	Tobacco, sunflower	First human protein expressed in plants
Hepatitis B virus envelop protein	Tobacco	First vaccine candidate expressed in plants
Hirudin	Canola	Thrombin inhibitor
Human serum albumin	Tobacco, potato	First full size native human protein expressed in plants
Lactoferrin	Rice, tomato	Antimicrobial activity
Rabies virus glycoprotein	Tomato	First example of an edible vaccine expressed in edible plant tissue
Secretory immunoglobulin A	Tobacco	First secretory antibody expressed in plant

cell culture in fermenters, or by the propagation of stably transformed plant lines in the field (Kamenarova, Abumhadi, Gecheff, & Atanassov, 2005). The feasibility of precise plant genetic manipulation, high-scale expression of transgene, rapid and easy scaling up, convenient storage of raw material and less concern of human or animal pathogens contamination during downstream processing have attracted biotechnologists to plant molecular pharming.

Transformation Methods

Molecular pharming relies on desired foreign genes which may be inserted or transformed into desired plants via a number of methods.

Stable Transformation

It is achieved by using *Agrobacterium*-mediated transformation or particle bombardment methods to integrate the desired foreign genes (Suslow, Thomas, & Bradford, 2002). In each method, the DNA sequence coding for the protein of interest and its promoter to make feasible its expression to a

particular tissue or developmental stage is ligated into the genome of the plant. Thus, when the plant is propagated, each plant will transmit foreign gene to its progeny and large numbers of plants having the transferred gene are readily generated. Alternatively, it is also possible to transform plastids in plant cells. Because genes in chloroplast genomes are not transmitted through pollen, recombinant genes are easier to contain and there is no risk of the transgene transfer to nontransgenic crops of same species or to those of related crops.

Recombinant Virus Vector

Transduction, a second method of engineering plants for protein expression; it utilizes a recombinant plant virus to transfer genes into plant cells. The DNA sequence coding for the desired protein is ligated into the genome of a plant virus which further allows infection of the host plant. As the virus replicates and spreads within the plant, many copies of the desired DNA are formed, and high levels of protein production are achieved in a short time. A limitation associated with this system is that green plants must be processed immediately after harvesting and cannot be stored.

Purification and Downstream Processing of Recombinant Proteins

Downstream processing is a process of recovery and purification of the desired recombinant protein from plants. Recovery usually consists of processing/fractionation of the plant tissue, solid–liquid separation, protein extraction, and concentration, whereas purification involves liquid–liquid extraction, immunoprecipitation, chromatography, membrane filtration, etc. Processing of leaves require special attention; it must be processed immediately after harvest or must be frozen to prevent degradation of recombinant proteins by proteases. The use of cell secretion systems is also a beneficial option as there is no need to disrupt plant cells during recovery, so the release of phenolic compounds are avoided. But the limitation of this system is that the recombinant protein may not be stable in the culture medium. Another way of protein recovery and purification is the use of affinity tags. Protein tags are removed after purification to restore the structure of the purified protein to its native form (Fischer, Stoger, Schillberg, Christou, & Twyman, 2004). When developing the strategy for heterologous protein production in plants, a proper consideration should be given to downstream processing feasibility of the recombinant protein to get maximum yield.

Biofertilizers

In strict sense, biofertilizers are not fertilizers. They are directly used for supply of nutrition to crop plants. Microorganisms such as bacteria, fungi, and blue green algae are largely used as biofertilizers. These organisms are

added to the rhizosphere of the plant to enhance their activity in the soil. They help the plants indirectly through better nitrogen fixation or improving the nutrient availability in the soil. Their mode of action differs and can be applied alone or in combination. By systematic research, efficient cultures are identified which are able to grow in given soil and climatic conditions. Such cultures are prepared at large scale in laboratory and distributed to farmers. For sufficient shelf-life, they are packed in carrier materials such as peat and lignite powder. Recently, use of biofertilizers is gaining momentum due to the increasing importance of maintenance of soil health, reducing environmental pollution, and the use of harmful chemicals in agriculture. For optimum plant growth, it requires nutrients in sufficient and balanced amount, but from the soil, only small portion of nutrients is released each year through biological and chemical processes. Therefore, fertilizers are used to supplement the nutrients which are already present in the soil (Chen, 2006). Microorganisms of biofertilizers play a vital role in accelerating the microbial processes such as controlling soil-borne diseases, improving the soil health, and soil properties. Biofertilizers can provide an economically viable option to small farmers for realizing the ultimate goal of increasing crop productivity. These are low-cost, effective and renewable sources of plant nutrients to supplement chemical fertilizers.

Biopesticides

Biotechnological tools provide solutions to common basic and applied problems that hinder use of insects' natural enemies as biological control agents. Commercially, biopesticides include microorganisms that control pests (microbial pesticides), naturally occurring substances (biochemical pesticides), and pesticidal substances derived from plants containing added genetic material (plant incorporated protectants). Recent registered global biopesticide products include bacteria (104 products, mostly are *B. thuringiensis*), fungi (12 products), viruses (8 products), nematodes (44 products), protozoa (6 products), and arthropod natural enemies (107 products) (Waage, 1996). These are used in agriculture for the purposes of insect control, disease control, nematode control, etc. Biopesticides are usually inherently less toxic than conventional pesticides.

Types of Biopesticides

Biopesticides are grouped into three different categories; microbial, plant derived, and biochemical pesticides.

Microbial Pesticides

Microbial pesticides consist of microorganism such as bacterium, fungus, virus, protozoan, or alga as its active ingredient. Microbial pesticides can target several different classes of pests but they are relatively specific for its

target pest. The most widely used microbial pesticides are varieties of the bacterium *B. thuringiensis* (Bt) which can control large number of insects in different crops. Bt encodes a protein that is lethal to specific insect pests. Microbial pesticides need careful monitoring of its application to ensure that they do not become harmful to nontarget organisms including humans.

Bacteria

Bacillus Thuringiensis (Bt)

B. thuringiensis is a ubiquitous, spore-forming, rod-shaped, Gram-positive bacterium that encodes large amounts of one or more toxic crystal proteins. *B. thuringiensis* was discovered by Ishiwaki in 1901 in diseased silkworm and was subsequently classified and named after its isolation from the gut of diseased flour moth larvae in Thuringberg, Ernst Berliner. These proteins are known as *Cry* protein, δ -endotoxin, or insecticidal crystal protein (ICP) which is toxic mainly to insect larvae in order Lepidoptera, Diptera, and Coleoptera but isolates with toxicity toward Homoptera, Orthoptera, Hymenoptera, Mallophaga, and against nematode, mites, protozoa have also been recently discovered (Lacey & Goettel, 1995). In addition to the *Bt* δ -endotoxin, a second class of protein which is lethal against certain insects such as the alpha-endotoxin, vegetative insecticidal proteins, and several secondary metabolites including Zwittermycin from *Bacillus cereus* strains may be used as a defense molecule against different insects. Various bacterial species and subspecies, especially *Bacillus*, *Pseudomonas*, etc., have been known to be used as biopesticides to control insect and plant diseases. Most important among these are insecticides which are based on several subspecies of *B. thuringiensis*. These subspecies include *B. thuringiensis* sp. *kurstaki* and *aizawai* having toxic activity against lepidopteran larval species, *B. thuringiensis tenebrionis* with activity against coleopteran adults and larvae, *B. thuringiensis israelensis* (*Bti*) with activity against mosquito larvae, black fly (simuliid) and fungus gnats and *B. thuringiensis japonensis* strain Buibui with activity against soil inhabiting beetles. Toxicity of *Bti* and other toxic strains is commonly attributed to the parasporal inclusion bodies (δ -endotoxins) which are produced during sporulation time. *Bt* and their subspecies produce different ICPs (δ -endotoxins), and their toxicity was determined. The structure of the *cry* genes and their δ -endotoxin product has been well characterized. The *cry* genes are carried on plasmids and belong to a superfamily of related genes. Classification of *cry* gene superfamily is based on size and sequence similarities. The sequence comparison indicates a large number of distinct families (*cry1*–*cry51*). Apart from sequence similarity, it is apparent that there are large differences in size among different *Cry* proteins, though they tend to cluster as either large (130 kDa) or small (70 kDa) protein. *Bt* produces crystalline proteins and kills several target insect pest species by

binding of the *Bt* Cry proteins to insect gut receptor after ingestion. These lethal toxins can damage the gut tissues by opening cation selective pore in midgut membrane leading to gut paralysis of insect larvae. After that, the infected larvae stop feeding and death results due to combined effects of starvation and damage of midgut epithelium. Preparation of *B. thuringiensis* spores or isolated crystals have now been used as organic pesticides. The isolated crystals have a limited persistence on foliage of a few days, whereas the spore preparations are effective for about 40 days on foliage and up to 2 years in soil.

Fungi

Although over 750 entomopathogenic fungal species were reported to infect insects, and the first registered mycoinsecticide was *Hirsutella thompsonii* which has been known to cause dramatic epizootics in spidermites. The mycoinsecticides *Verticillium lecanii* and *Paecilomyces fumosoroseus* are recently registered for control of whitefly, thrips, aphids, and spider mites. Insect fungi that have much broader host range are *Beauveria bassiana* and *Metarhizium anisopliae* which are effective against homopteran and lepidopteran greenhouse insects as well as coleopteran and lepidopteran field insects (Flexner & Belnavis, 1998).

Baculovirus

Baculoviruses are present in arthropods mainly in insects. Baculoviruses are highly pathogenic group of viruses and have been used effectively in their native form as biocontrol agents against various potential insect pests, but the application of baculoviruses is less common in the field of agriculture and horticulture. Baculoviruses have been isolated primarily from order lepidoptera and they only cause mortality in the larval stage. As larvae ingest baculoviruses, it initiates effective infection. After ingestion they spread systematically throughout the body through the midgut of larvae. First, introduction of baculovirus into the environment leading effective suppression of a pest occurred accidentally before the World War II. Nucleopolyhedroviruses and Granulosis viruses are the examples of baculoviruses used as pesticides.

Plant-Derived Insecticides

Since ancient times, natural compounds from plants (Table 2.2) were used to give defense from insect pests. Plants and some insects have coexisted on the earth for almost 3.5 million years, which has allowed lots of time for both to develop strategies for offense and defense. Plants have developed several strategies to protect them by predators; for example, they developed compounds that are highly toxic to insects. Several single-gene products of plant have been

TABLE 2.2 Some Plant Products Registered as Biopesticides

Plant Product Used as Biopesticides	Target Pest
Limonene and Linalool	Fleas, aphids, mites, fire ants, several types of flies, paper wasp, and house crickets
Neem (plant parts)	Variety of sucking and chewing insect
Pyrethrins/Pyrethrum	Ants, aphids, fleas, flies, and ticks
Rotenone	Leaf feeding insects, e.g., aphids, certain beetles (Asparagus beetle, Colorado potato beetle, leaf beetle, etc.)
Ryania	Caterpillars (European corn borer, corn earworm) and thrips
Sabadilla	Squash bug, Harlequin bug, thrips, caterpillars, leaf hoppers, and stink bugs

identified to provide resistance against insect damage and have been successfully transferred to other plants. Carbohydrate-binding proteins such as lectin and lectin-like proteins are copious in seeds and storage tissue of plants, and these proteins are used as defensive tools by plants against insect particularly for homopteran plant pests such as aphids, leaf hoppers, and plant hoppers.

Neem

It was estimated that more than 2400 plant species throughout the world are currently known to confer pest control properties. The important species with insecticidal property includes neem, onion, sweet flag, garlic, custard apple, holy basil, pyrethrum, derris, common lantana, black pepper, and common ginger. Seeds or seed kernels provide the largest amount of insecticidal preparations. Neem is generally known to be most effective against the soft-bodied, immature stages of plant pests including thrips, mealybugs, whiteflies, and various caterpillars (Weinzierl, 1998). Its broad activity against plant pests, its virtual nontoxicity to mammals, beneficial insects and environment make it an extremely attractive insecticide. Neem is a naturally derived material from Neem trees (*Azadirachta indica*) native to India. It is reported that out of 450–500 insect pest species assessed for susceptibility, 413 insect pest species are susceptible at various concentrations of neem products. Among the isolated neem constituent, limonoids (azadirachtin) are effective in insect growth regulatory activity. Azadirachtin indirectly kill pests by altering the life processing behavior in such a way that the insect can no longer feed, breed, or complete its metamorphosis. More specifically, azadirachtin inhibits metamorphosis by interfering with biosynthesis or metabolism of juvenile hormone ecdysone. Plant-derived insecticides may be

in the form of dust or powder of crude preparations that may be used in full strength or diluted form. Some preparations are the water extracts or organic solvent extract of insecticidal components of plants. More than 15 complex chemicals having repellent, antifeedant, and insect growth have been known. Azadirachtin affects the physiological activities of insects and does not affect other biocontrol agents.

Biochemical Pesticides

Biochemical pesticides are believed to be closely related category of conventional chemical pesticides. Conventional pesticides are synthetic chemical products used to kill or inactivate the pest. Biochemical pesticides are the substances which interfere with growth or mating of insect pest, such as plant growth regulators, or substances that repel or attract pests, such as pheromones. A large number of pheromones were used to interfere or interrupt the life cycle of the insect by preventing mating (Suckling & Karg, 1998). The active ingredient can be a single molecule or a mixture of molecules, such as a naturally occurring mixture comprising of a plant essential oil, or a mixture of very structurally similar molecules called isomers in the case of insect pheromones. Some essential oils work as repellents and their mode of action would be like a fragrance which is naturally occurring substances that control pests by nontoxic mechanisms. The production of total world biopesticides is increasing and, therefore, demand and use are also increasing.

BIOTECHNOLOGICAL TOOLS FOR LIVESTOCK IMPROVEMENT

Biotechnology is thought to be synonymous with recombinant DNA techniques or genetic engineering. However, in practice, biotechnology includes not only genetic engineering but also some of the older and closely related tools such as manipulation of reproductive processes. Thus, using biotechnological tools scientists manipulate not only the genetic make-up of living organisms but also process or factors which preexist in living organisms. Biotechnological tools are crucial to enhance animal production and to conserve the indigenous animal genetic resources. These tools are given in the following sections.

Marker-Assisted Selection

Traditionally, livestock improvement was aimed on the selective breeding of individual animals with superior phenotypes without knowing which genes are being selected. This led to the development of livestock with characteristic phenotypes that might be classified as distinct breeds (Williams, 2005).

Unfortunately, the phenotype is an inadequate predictor of the breeding value of livestock in light of the fact that it could be sex specific or manifest after the selection phase in the breeding life of an animal. Likewise, the determination of negative associations between genes on the basis of phenotype is poor. The main aim of livestock breeding is to maximize response to selection per generation. To achieve this selection on the basis of DNA, markers offers a way round some of these limitations, as DNA markers can be tested at any age and can be measured in either sex. However, in recent years, information on the organization and functioning of genomes of livestock are getting to be accessible, which can be utilized in breeding programs for the genetic improvement and selection of livestock. The addition of genomic information to phenotypic information to enhance the selection response to the traditional breeding method for rapid genetic gains is known as MAS. The idea behind MAS is to use genetic markers rather than the phenotypes. MAS is indirect selection process where a trait of interest is selected on the basis of marker linked to it in place of trait itself (Wakchaure et al., 2015a). Some traits are controlled by single genes (e.g., hair color), whereas most traits (such as milk yield and growth rate in animals) considered in animal genetic improvement programs are quantitative traits which are controlled by many genes and environmental factors (Zaman, 2013). The genetic markers are used to identify loci or chromosomal regions that affect single gene traits and also quantitative trait locus (QTL). This has provided opportunities to enhance genetic improvement and selection (MAS) in livestock breeding, in particular for traits that are difficult to improve by traditional selection. Molecular techniques allow detection of the existence of polymorphisms through marker among individuals in the population for specific regions of the genome. These polymorphisms can be used to create genetic maps of the species on the basis of markers. These genetic maps are used to evaluate differences between markers in the expression of particular traits that can be detected by testing for statistical associations between marker variants and any trait of interest. A number of genetic markers are available to study the genetic structure of traits and its use in MAS.

Genetic Marker

Genetic markers (DNA markers) are identifiable DNA sequences found at specific locations of the genome and transmitted from one generation to the next by the law of inheritance. These markers should not be considered as normal genes because they usually do not have any biological effect (Moniruzzaman, Khatun, & Mintoo, 2014). Genetic markers are used for the identification of locations in the genome. These markers can be used to track the inheritance of simple traits controlled by a single gene or complex traits controlled by many genes. There are two types of markers: (1) linked and (2) direct, which can be considered in the genetic studies. The linked

markers are sufficiently close to the trait genes on the chromosome and alleles at the marker and the trait gene are inherited together. The direct marker is a functional polymorphism in the gene that controls variation in the trait. The genetic markers relate alleles for quantitative characteristics as well as helping to understand the quantitative variations and their role in genetic improvement and selection of livestock. There are different classes of genetic markers as described below.

Restriction Fragment Length Polymorphism

A gene with Mendelian inheritance could act as a marker for the segregation of a gene involved in the expression of a quantitative trait. Restriction fragment length polymorphism (RFLP) analysis was developed to visualize the differences at the level of the structure of the DNA. It is based on the use of bacterial restriction enzymes that cut the DNA molecules at highly specific recognition sequences. Variations at restriction enzyme recognition sites in the genome could be identified by digestion of genomic DNA with a restriction enzyme and observing the pattern of formed DNA fragments by gel electrophoresis (Williams, 2005). However, many fragments are produced after digestion of genome with a restriction enzyme, and it is due to the large number of recognition sites within the genome. Therefore, the DNA fragments of differing sizes produced by a genomic digest are separated by gel electrophoresis and transferred onto a nylon matrix (Southern blot). Polymorphisms within a particular gene are then identifying by hybridization with a radioactive or chemiluminescent-labeled gene-specific probe. This approach allows each locus to be studied separately and is a powerful way to examine variations at a particular point in a given gene.

Microsatellite Markers

The microsatellite markers are polymerase chain reaction (PCR)-based markers. These are used to identify alleles of QTL via association between the markers and phenotype. Microsatellite loci contain multiple copies of tandem repeat sequence. The tandem repeats are usually simple dinucleotides, and the number of repeat units varies between individuals resulting in a large number of alleles for a given locus. The relatively large number of alleles at microsatellite loci shows high degree of polymorphism and their amenability to PCR amplification make their use as location markers for use in genome mapping and genetic studies (Moniruzzaman et al., 2014).

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) are single base-pair variations in the DNA sequence due to insertions or deletions. SNPs are more frequent and occur at high frequency in both noncoding and coding regions of the genome. SNPs within coding regions of genome may have no effect on the protein coded by the gene or may result in a causative mutation that result in

a single amino acid change in the protein sequence. The latter are most likely to be the functional polymorphisms that are responsible for the phenotypic variation in traits (Williams, 2005). The genes involved in both disease and in normal biological processes can be identified with the help of analysis of DNA sequence variation.

Reproductive Technologies

Livestocks account for the part of economy of farmers in terms of milk, meat and wool production, etc. (Martinez, 2012). Considering this, the utilization of reproductive technologies has, therefore, evolved in livestock production to allow producers to have further control over reproduction. These technologies are also known as assisted reproductive technologies which allow the genetic improvement of livestock through increasing the selection differential with use of the genetically superior individuals. Reproductive biotechnological tools are used for reducing the generation interval and increasing the frequency of offspring production from selected genetically superior livestock (Chakravarthi & Balaji, 2010). These technologies are also helpful in the identification of better sires and dams, herd genetic progress, and reduction in production costs. The reproductive biotechnological tools are in vitro fertilization (IVF), artificial insemination and embryo transfer (ET).

In Vitro Fertilization

The IVF tool evolved from the interest in developing a system to produce embryos completely in the laboratory (Bertolini & Bertolini, 2009). It is a valuable tool to assist genetic selection strategies and breeding plans for livestock production systems. It can be utilized to improve pregnancy rates in herds with low fertility or certain reproductive breakdowns such as ovulation and fertilization failures or reproductive-tract blockage (Debnath, Sinha, Khushboo, Vedamurthy, & Mamatha, 2013). The IVF technique becomes an increasingly important tool which has been employed commercially for assisted production in livestock due to its low cost, combined with the increase in transferable embryos, and pregnancies per estrous cycle. IVF allows for more efficient use of semen preserved from decedent genetic lines or highly valuable semen. It improves the efficiency of sex-sorted sperm to produce desired gender offspring.

IVF is accomplished by generating embryos from donors and transferring them into recipients with less genetic merit. The procedure of IVF varies among different species. In general, it involves four major steps (Fig. 2.2):

1. The collection of immature oocytes (unfertilized eggs)
2. The maturation of immature oocytes
3. The fertilization of mature oocytes
4. The culture of embryos

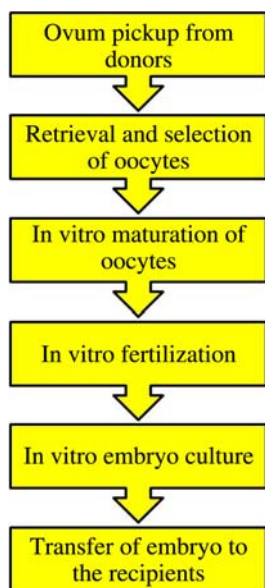


FIGURE 2.2 General procedure of in vitro fertilization.

The immature oocytes (unfertilized eggs) are collected from abattoir-derived ovaries or from live donors using ovum pick-up aspiration (Velazquez, 2008). The collected oocytes are taken into the laboratory where it is rinsed and then retrieved, counted, and graded with the help of a microscope. Once the oocytes have been processed, they are placed into dishes containing media designed to mature them. The dishes containing evaluated oocytes are placed in an incubator for 18 to 24 hours where the maturation process takes place. After incubation, semen is added to the dishes containing the matured oocytes which lead to the fertilization. For successful fertilization of oocytes, good sperm preparation is the essential and crucial step. The media used for IVF must be capable of supplying the nutrients and chemical signals to increase sperm motility and induction of capacitation (Wakchaure, Ganguly, Para, Praveen, & Qadri, 2015b). Capacitation involves modifications of the sperm plasma membrane, which make it to become unstable and to undergo vesiculation with the outer acrosomal membrane. It facilitates the fusion of gametes and the beginning of embryonic development. After fertilization, the oocytes are placed in the incubators for the next 7 days for the development of fertilized oocytes into embryos. These embryos can be evaluated under a microscope and quality graded. The graded embryos (blastocysts) are implanted into the synchronized recipient who is in the correct receptive stage of the estrus cycle or are frozen for future use.

Although in vitro production has been achieved in farm animals, further investigation is required for refining such protocols to establish an effective IVF system for use with a broad range of livestock.

Artificial Insemination

Artificial insemination (AI) is a very effective reproductive technique widely used for livestock breeding around the world. It can be used as a tool to increase conception rate in animals that have fertility problems. AI can be a very useful technique in propagating genetic material derived from genetically superior livestock. It was the first-assisted reproductive technique applied to control and enhances reproduction efficiency, and in addition genetic improvement of livestock. AI is the manual placement of semen collected from a male animal in the reproductive tract of a female animal by a method other than natural mating in order to get the female impregnated (Morrell, 2011). AI with fresh or frozen semen is the most common method of breeding in the majority of domestic livestock species such as dairy cattle, pigs, poultry (turkeys, fowls, ducks) goats, sheep, horses, and rabbits. The technique of AI depends on species, type of semen, breeding system, availability of equipment, and expertise. The different methods of semen deposition like intravaginal (dogs), intracervical (sheep), transcervical intrauterine (cattle, horses, dogs, sheep), transcervical deep horn intrauterine (cattle, horses, pigs), and laparoscopic (sheep) are available for different animals (Heise, 2012). The breeding of dairy cattle using AI is an effective way to increase milk production. The good quality semen from superior animal is used for genetic improvement of livestock. The steps involved in AI are as described below (Morrell, 2011).

Collection of Semen

The semen is collected by a rubber device known as artificial vagina in the case of most domestic animals. This device is shaped and constructed to resemble the animal vagina. For collection of semen, the device is placed at the same angle as the penis. The penis feels the artificial vagina and thrust to ejaculate. The semen is collected and taken into the laboratory for processing.

Processing of Semen

In laboratory, the collected semen undergoes a series of international standard tests for sperm quantity, volume (number of live sperm), and quality (sperm of the correct shape and size). A semen extender is added in the collected semen to dilute toxic elements in seminal plasma, to provide nutrients for the spermatozoa during in vitro storage and to buffer their metabolic by-products.

Semen Preservation

Semen is used either immediately after collection and processing, or it is stored for future use. The storage of semen is in two forms.

1. **Storing at reduced temperature:** The reduced temperature helps to extend sperm life by slowing their metabolism as well as by inhibiting bacterial growth.
2. **Frozen at very low temperature (cryopreservation):** In this form, semen is blended with a protective solution containing lipoproteins, sugars, and a cryoprotectant such as glycerol and preserved in liquid nitrogen at -196°C . The protective solution helps to preserve membrane integrity of spermatozoa during the processes of cooling and rewarming.

Deposition of Semen in the Female

The success of AI depends on depositing semen in female tract at around the time of ovulation so accurate detection of estrus is crucial. Some livestock may show well-developed estrous behavior like dairy cows. In ruminants and primates, semen is deposited in the vagina, whereas in pigs, dogs, camels, and horses, semen deposition is intrauterine. In most species, the semen is deposited in the uterus by insemination catheter through the cervix.

Embryo Transfer

There are estimated billions of sperms produced by each bull and 150,000 potential eggs or ova in the cow. Only a fraction of the reproductive potential of an outstanding individual could be utilized by natural breeding. The average cow will have one calf per year and average herd bull will sire 15 to 50 calves per year. It is possible to exploit the vast number of sperms produced by a genetically superior bull with the help of AI techniques, but the reproductive potential of the female has been largely unutilized (Selk, 2007). Under normal management programs, a cow produces an average of eight to ten calves in her lifetime. The number of offspring produced by a genetically important cow can be greatly increased by ET technique. ET is a process by which an embryo is collected from a genetically superior donor female and afterward transferred into a recipient female where the embryo completes its development. Through the use of ET, a genetically superior female produces more offspring than she could by natural reproduction. The increased number of offspring thus maximizes the donor female's genetic abilities. ET is used in several species of livestock like cows, horses, goats, and sheep. The ET technique includes some or all of the following steps (Selk, 2007) (Fig. 2.3).

Selection of Donor Female

The donor female for ET is selected on the basis of their genetic merit, reproductive performance and progeny performance. The potential donor cow must be healthy and reproductively sound to produce maximal results.

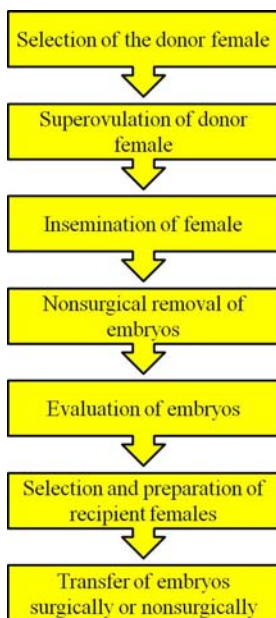


FIGURE 2.3 General procedure of embryo transfer.

Superovulation of Donor Female

Superovulation refers to the release of multiple oocytes (eggs) at a single estrus period. The basic principle of superovulation is to stimulate extensive follicular development through treatment with gonadotropins. The donor female for ET are treated with follicle-stimulating hormone to induce the maturation and ovulation for greater than normal number of oocytes.

Insemination

The superovulated donor female releases a large number of eggs from the multiple follicles on the ovary. Therefore, many ET technicians choose to inseminate the donor several times during and after estrus to achieve optimal fertilization.

Recovery of Embryos and Evaluation

A specially designed small-synthetic rubber catheter (Foley catheter) is used to collect the embryos nonsurgically. The catheter is inserted into the vagina and through the cervix of the donor cow. A special medium from the catheter is flushed into and out of the uterus to harvest the embryos 7 or 8 days after estrous. The flushed fluid is collected in a special container, and the embryos are filtered from the collected fluid. The collected embryos are evaluated and graded on the basis of quality (shape, color, texture, and size)

using a stereoscopic microscope. Embryos are also classified on their stages of development.

Selection of Recipient Females

The recipient females to be implanted with the collected embryos should be healthy, good body condition, and reproductively sound. To maximize the success rate of the transfer, recipient females need to be at the same stage of the estrous cycle as the donor when she donated the embryos. Estrous synchronization is typically used to manipulate recipients so that they are at the correct stage of the estrous cycle. It is important to establish a uniform uterine environment for the embryo.

Transfer of Embryos

The transfer gun or insemination rod is used for transfer of embryo into the recipient females. The ovaries of the recipient are palpated rectally to determine the presence and location of ovary which has ovulated. The transfer of the embryo into the recipient female requires loading of the embryo into an insemination straw. An insemination rod is passed through the recipient's cervix and into her uterus. The embryo is gently expelled into the uterine horn that is on the same side of the ovary with an active corpus luteum as the ovulated ovary.

Genetic Engineering and GM Animals

Traditionally, genetic improvement of livestock has been accomplished with the help of quantitative genetics and animal breeding methods. The modern biotechnology provides new avenues for genetic improvement in production animals with molecular-based technologies such as genetic engineering (Murray & Anderson, 2000). Genetic engineering is the manipulation and modification of organism's genome using modern DNA technology for many beneficial purposes. It involves the transfer of foreign DNA/genes within and across the species boundaries to produce improved or novel organisms. Genetic engineering in livestock production has increasing number of practical benefits, such as in the production of transgenic animals or GMOs.

GM Animals/Transgenic Animals

A transgenic animal or GM animal or GE animal is an animal in which foreign DNA (transgene) has been incorporated from other organisms in a process called transgenesis. Transgenes are produced by genetic engineering techniques including isolation, cutting and transfer of specific DNA pieces, corresponding to specific genes (Klug & Cummings, 2002). The purpose of adding a transgene to livestock genome is to have the organism produce a protein or set of proteins that normally they would not produce. Production

of transgenic livestock provides a method to rapidly introduce new genes into livestock without crossbreeding. The transfer of gene is a beneficial way of altering or modifying the genetic make-up of livestock in a much more directed way as compare to conventional breeding and selection methods. The use of these methodologies will have a great impact toward improving the efficiency of livestock.

Production of GM Livestock/Transgenic Livestock

The following sequential steps are generally adopted for the production of transgenic livestock irrespective of species (Bagle, Kunkulol, Baig, & More, 2013):

- Identification and construction of foreign gene (transgene)
- Introduction of transgene directly into the pronucleus of fertilized egg by different methods
- Implantation of these inoculated fertilized eggs into surrogate mothers or foster mothers
- Bringing the developing embryo to term, proving that the transgene has been steadily and heritably incorporated into the DNA in all of their cells of at least some of the newborn offspring derived from the implanted eggs
- Demonstrating that the gene is regulated well enough to function in its new environment

In order to produce GM livestock, different methods are used for the transfer of foreign DNA (transgene) according to the animal species.

Microinjection

Microinjection is the most widely applied method for gene transfer in animals used to produce GE livestock (Murray & Anderson, 2000). It involves the microinjection of foreign DNA into the pronucleus of a fertilized ovum. In this method, the superovulated donor female is mated with fertile male. The resulting fertilized eggs are collected from superovulated donors and microinjected with transgene into male pronuclei. Microinjection equipments include microscopes and micromanipulators. The embryos carrying the transgene are then transferred to hormonally prepared recipient females (foster mother or surrogate mother) using surgical or nonsurgical operations.

The reproduction rate of bovine is slow and the number of embryos generated by superovulation is low. Hence, the success of microinjection appeared accessible in these animals only if embryos were prepared in vitro. The oocytes collected from donor females is matured and fertilized in vitro condition. The fertilized egg is then microinjected with transgene into male pronuclei before the sperm and egg pro-nuclei are able to fuse. The fertilized egg is allowed to in vitro development to the blastocyst stage. The embryos are then implanted into the uterus of a recipient female (Fig. 2.4).

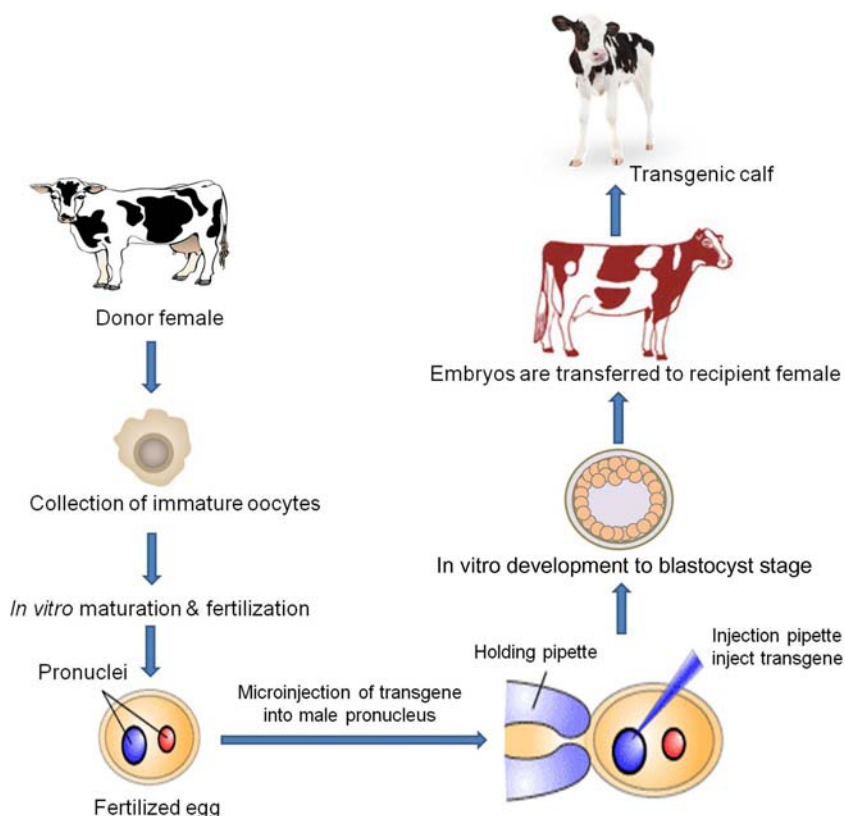


FIGURE 2.4 Production of genetically modified livestock by microinjection method.

Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer is a technique for cloning. This process involves collecting an egg cell from an animal and a somatic or nonreproductive cell from the animal that is to be cloned (donor cell). The nucleus is removed from an egg cell. The enucleated egg is transplanted from a donor somatic or nonreproductive cell. The resulting embryo is implanted into the uterus of a surrogate mother by surgical or nonsurgical procedure to develop and produce cloned offspring. The offspring is essentially a genetic clone of the animal from which the donor nucleus was obtained (Fig. 2.5).

Sperm-Mediated Gene Transfer

The sperm incubated in the presence of foreign DNA before being used for fertilization has the ability to transfer the foreign DNA into the oocyte. The finding suggested about the sperm-mediated gene transfer strategy for animal transgenesis. In this method of transgenesis, sperm cells free from seminal

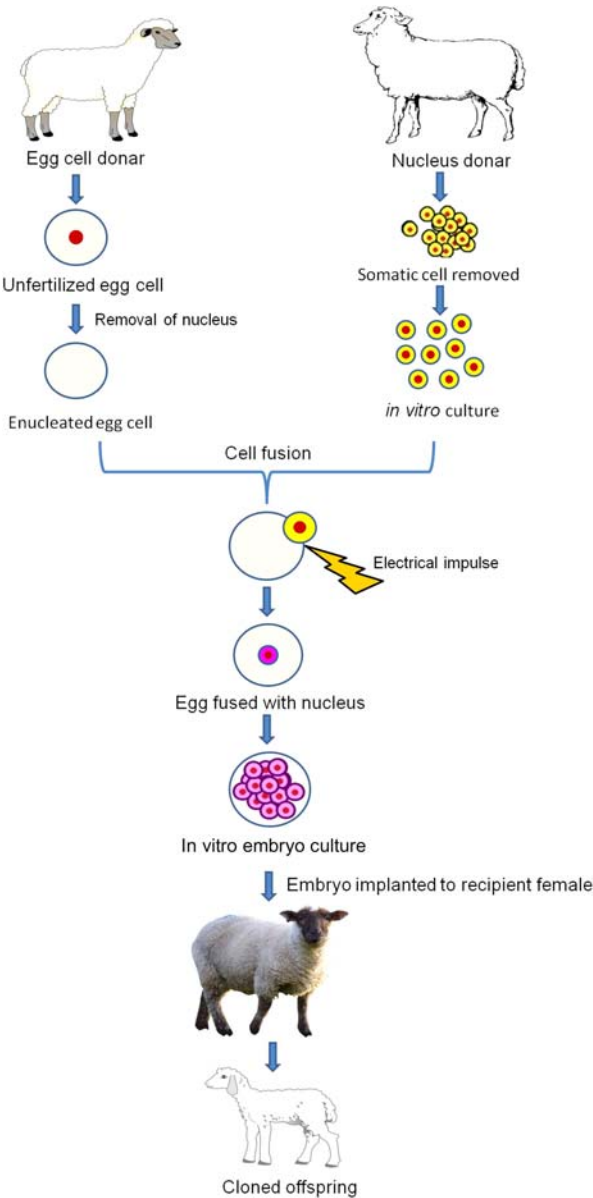


FIGURE 2.5 Production of genetically modified livestock by somatic cell nuclear transfer.

plasma are suspended in suitable medium. The sperm cells are incubated with foreign DNA (transgene). The resultant transgene carrying sperms are now used to fertilize eggs via AI or IVF leading to the generation of transgenic animal. The augmentation technique such as electroporation and

liposomes are also used for improving the association and internalization of foreign DNA into spermatozoa.

Retroviral-Mediated Gene Transfer

Retroviruses are animal viruses containing two identical single stranded RNA genomes. These viruses are used as vectors to transfer genetic material into the host cell. When cells are infected by retroviruses, the RNA genome are converted to double strand DNA by reverse transcription and integrated into the host cell genome (Rajoriya, Rajoriya, & Kumar, 2013). For production of transgenic livestock, the transgene construct are packaged into mature viral particles with the help of packaging cell line and used to transfer it into oocytes or one-cell embryos.

Applications of GM/Transgenic Livestock

There are various potential applications of transgenic technology in producing new or altered varieties of agriculturally important livestock.

Improved Milk Production and Composition

The quality and quantity of milk produced by livestock can be improved by utilizing transgenic technology. The main target for improving the milk composition is casein protein (Niemann, Kues, & Carnwath, 2005). Transgenic cattle with extra copies of the casein gene could result in increased amount of casein in milk that increase the value of milk for manufacturing and industrial purposes such as in the production of milk-based products like cheese, yoghurt. Transgenic alteration of milk composition has the potential to enhance the production of certain proteins and growth factors that are deficient in milk.

Improved Growth Rate and Carcass Composition

It is possible to manipulate the known growth factors, growth factor receptors and growth modulators with the help of transgenic technology. Transgenic animals with transgene (growth hormone and insulin-like growth factors gene) constructs have enhanced growth rate and improved quality of food (Asaye, Biyazen, & Girma, 2014). The introduction of these genes have been expressed at different levels in transgenic animals which play a major role in modification of growth and body composition for increased meat production by stimulating growth rates.

Increased Disease Resistance

An important application of transgenic technology in agriculture is the potential to enhance disease resistance capacity of livestock by introducing specific genes of immune system. Transgenic strategies to increase disease

resistance of livestock include the transfer of major histocompatibility complex genes, T-cell-receptor genes, immunoglobulin genes, or specific disease-resistance genes which influence the immune response (Niemann et al., 2005).

Transgenic Livestock as a Walking Bioreactor

Animal pharming, the way toward utilizing transgenic livestock to produce human drugs, is asserting some authority in a remunerative world market. The potential use of transgenic livestock is the production of recombinant and biologically active proteins in the mammary gland. The preferred site for production of these proteins is mammary gland of livestock because large quantities can be extracted and purified (Rudolph, 1999). Using transgenic livestock as bioreactors is cost-effective and advantageous because animals naturally carry the cellular mechanisms needed to produce complex proteins. Several novel therapeutic proteins have been derived from the transgenic livestock (Bagle et al., 2013) (Table 2.3).

Molecular Diagnostic Tools and Animal Health

Molecular diagnostics is a collection of techniques used to analyze biological markers in the genome and proteome by applying molecular biology to medical testing. Molecular techniques are used to diagnose and monitor disease, detect risk, and decide which therapies will work best for individual animal health. Diagnosis is important in the control and prevention of endemic livestock diseases in the developing countries. Molecular diagnostic tools are commonly used for the diagnosis of infectious disease by veterinary practitioners. These tools offer low cost diagnostic decision-support system and leads to the upgradation in clinical area by veterinary and animal health

TABLE 2.3 Products from Transgenic Livestock	
Transgenic Animals	Proteins
Chickens and eggs	Human serum albumin (HSA), insulin, vaccines, MAbs interferons, cytokines
Goats	tPA (tissue plasminogen activator), ATryn (recombinant human antithrombin III), monoclonal antibodies (MAbs), Ig fusion proteins
Pigs	Recombinant antithrombin III (rATIII), Factors VIII and IX, protein C, human milk protein, recombinant HAS
Sheep	alpha-1-antitrypsin, fibrinogen, human Factor VII, Factor IX, activated protein C

professionals in several countries across the world. In the last few decades, veterinary diagnosticians have incorporated new molecular techniques (Table 2.4) such as the DNA microarray, polymerase chain reaction, enzyme assay, electrophoresis, biosensor, DNA probes, flow cytometry, Western

TABLE 2.4 Summary of Molecular Diagnostic Tools and Its Applications

Diagnostic Tools	Applications
Biosensors	Detection of the target pathogen or a disease-specific antibody
Capillary electrophoresis	Food analysis, quantitative chemical analysis of food additives, biochemical analysis of protein composition
DNA microarray technology	Investigation of diseases of unknown etiology
DNA probes	Detection of target DNA sequence by hybridization
Enzyme-linked immunosorbent assay	Detection of various genetically modified (GM) proteins of plants and animals
Flow cytometry	Detection and physiological assessment of microorganisms in drinking water, marine environments, food and fermentation processes. Characterization of genome sizes of fungal and oomycete populations, multiplexed pathogen detection and the monitoring of the viability, culturability and gene expression of plant pathogens
Immunofluorescence	Detection of antigen/protein using the specific antibodies. Rapid identification of nodule bacteria either in very early infection stages or from mature nodules
Microchip electrophoresis	Rapid and sensitive determination of herbicide (glyphosate and glufosinate) residues in agriculture, clinical diagnosis of protein and DNA
Polymerase chain reaction	Products development, grain processing, identification of fishery products, cultivar identification of rice and quantification of fungal plant pathogens in wheat and barley
Pulsed field gel electrophoresis	Subtyping of many pathogenic bacteria, cloning of large plant DNA, construction of physical maps and genetic fingerprintings
Radioimmunoassay	Detection of circulating antibodies to Histoplasma mycelial, Histoplasma yeast, and Blastomyces yeast antigens. Measure the progesterone in blood or milk from cows for studies on reproductive efficiency
Western blotting	Study of plant virus characteristics, virus particle-protein interactions, electrophorotype formation, and strain separation

blot, etc. for diagnosis of livestock diseases. Although conventional diagnostic tools are still in use, the new molecular tools have enlarged the scope of veterinary diagnostics and provide powerful new approaches that enable the rapid and specific diagnosis of livestock diseases.

Vaccine Technology

A vaccine is a biological preparation that provides active acquired immunity to a particular disease. Since the discovery of the first vaccine by Edward Jenner in 1796, various approaches and technologies have been employed to develop safe and efficacious vaccines that produce heightened immune responses after a single dose which provides cost-effective and long-lasting immunity to the host (Levine, 2011) (Fig. 2.6). Current vaccine technologies are based on either whole organism (live attenuated or killed microorganism), microbial components (peptide, protein, toxoid, and recombinant), or microbial genetic components (DNA or RNA) technology-based vaccines. Other approaches employ chemically synthesized adjuvants or adjuvant-like molecules that can attach on antigens and provide better immunity.

Vaccine may be a bacterium, virus (Table 2.5), antigen or toxin, a recombinant vector or a synthetic peptide or DNA, and all of them can be introduced in various forms (Gould & Venugopal, 1994). The aim of vaccination is to prevent the onset of the diseases by calling an early immune response to a pathogen, without damage to health, which causes the body to be prepared for a possible infection.

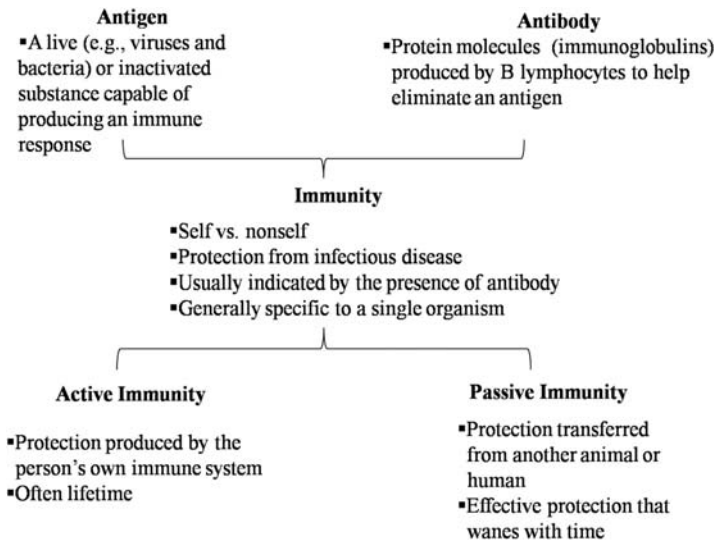


FIGURE 2.6 Component of immune system and their response.

TABLE 2.5 Bacterial and Viral Vaccines

Bacterial Vaccines			
Bacterium	Diseases or Conditions	Vaccine(s)	Vaccine Type
<i>Bacillus anthracis</i>	Anthrax	Anthrax	Live attenuated
<i>Bordetella pertussis</i>	Whooping cough	DPT	Subunit, Killed
<i>Clostridium tetani</i>	Tetanus	DPT	Toxoid
<i>Corynebacterium diphtheriae</i>	Diphtheria	DPT	Toxoid
<i>Haemophilus influenzae</i> type B	Epiglottitis, meningitis, pneumonia	HIB	Conjugate
<i>Mycobacterium tuberculosis</i>	Tuberculosis	BCG	Live attenuated
<i>Neisseria meningitides</i>	Meningococcal meningitis	Meningococcal	Polysaccharide
<i>Salmonella typhi</i>	Typhoid fever	Typhoid	Polysaccharide
<i>Streptococcus pneumoniae</i>	Pneumococcal pneumonia	Pneumococcal conjugate	Conjugate
<i>Vibrio cholera</i>	Cholera	Cholera	Killed
Viral vaccines			
Virus	Diseases or Conditions	Vaccine(s)	Vaccine Type
<i>Hepatitis A virus</i>	Hepatitis A	Hepatitis A	Killed
<i>Hepatitis B virus</i>	Hepatitis B	Hepatitis B	Subunit
<i>Influenza virus</i>	Influenza	Influenza	Killed
<i>Japanese encephalitis virus</i>	Japanese encephalitis	Japanese encephalitis	Killed, Live attenuated
<i>Measles virus</i>	Measles	MMR & MMRV	Live attenuated
<i>Mumps virus</i>	Mumps	MMR & MMRV	Live attenuated
<i>Polio virus</i>	Poliomyelitis	Polio	Live attenuated, Killed
<i>Rotavirus</i>	Rotaviral gastroenteritis	Rotavirus	Live attenuated
<i>Rubella virus</i>	Rubella	MMR vaccine, MMRV	Live attenuated
<i>Varicella zoster virus</i>	Chickenpox, shingles	Varicella, shingles, MMRV	Live attenuated

DPT, diphtheria, pertussis, and tetanus; BCG, Bacillus Calmette–Guerin; MMR, measles, mumps, and rubella; MMRV, measles, mumps, rubella, and varicella; HIB, *Haemophilus influenzae* type B.

Properties of some ideal vaccines are as follows:

1. Provide long lasting immunity
2. Should induce both humoral and cellular immunity
3. Should not induce autoimmunity or hypersensitivity
4. Should be inexpensive to produce, easy to store, and administer
5. Vaccines must also be perceived to be safe

Whole Organism Vaccine Technology

These types of vaccines are prepared by live attenuation, killing, or inactivation of pathogens.

Live-Attenuated Vaccine

Attenuation can be achieved by chemical or heat treatment or culturing the pathogen under adverse conditions. The aim of attenuation is to completely eliminate the pathogen's virulence. However, it does not lose its properties in generating an immune response of the host. Today, researchers weaken or attenuate pathogens by growing them in a series of nonhuman cell cultures and selecting for those with lowered capability to reproduce in humans. Common live, attenuated vaccines are *Bacillus Calmette–Guérin* (BCG); measles, mumps, and rubella (MMR); chickenpox; and flu vaccines. The advantage of live attenuated vaccines is that they are very effective in inducing full protection against their diseases. However, some disadvantages of live vaccines are as follows:

1. The viruses are still live and can mutate to a more dangerous form
2. Peoples with weak immune systems get sick even from the weakened form of the virus
3. To remain effective, needs constant refrigeration

Inactivated (Killed) Vaccine

Inactivated (killed) vaccines are consisting of virus particles, bacteria, or other pathogens that have been grown in controlled culture condition and then killed either by heat or chemicals (mostly formaldehyde) to reduce their virulence and, thus, prevent infection from the vaccine. Attenuated vaccines are often preferable for generally healthy people because a single dose is often safe and very effective. Attenuated vaccines have some more advantages in comparison to inactivated vaccines (Table 2.6). However, some people cannot take attenuated vaccines because the pathogen poses too much risk for them especially for elderly people or people with immunodeficiency. For those patients, an inactivated vaccine can provide protection. Some of the viral and bacterial inactivated vaccines are polio (Salk vaccine), influenza and typhoid, cholera, plague, and pertussis vaccines.

TABLE 2.6 Comparison Between Live and Inactivated Vaccines

Property	Live (Attenuated)	Inactivated (Killed)
Cost	Lower	Higher
Administration	Oral	Parenteral
Adjuvant	Not needed	Needed
Stability	Poor	Good
Reversion	Possible	Absent
Immunity	Mucosal immunity present	Mucosal immunity absent
	Antibody mediated and cytotoxic T cells	Antibody mediated
	Long lasting	Short lasting

Subunit Vaccine Technology

Subunit vaccines, like inactivated whole-cell vaccines, do not contain live components of the pathogen, instead they contain isolated pathogen's protein and presented to immune system for their response against these pathogens. Through the protein engineering technologies, number of subunit vaccines can be increased by introducing the subunit coding sequence in to host body that will produce it in large quantities. Mutations can then be introduced that reduce or eliminate the toxicity or alter other properties of the molecule (Pingoud & Jeltsch, 2001), along with the use of many other tools of genetic engineering. One method of production of subunit vaccines involves isolation of subunit coding protein from a virus and administering this by itself. Another method of subunit vaccine production involves putting an antigenic gene from the targeted virus or bacterium into another virus or yeast vectors (hepatitis B vaccine) or attenuated bacterium vector to make a recombinant virus or bacteria vaccine. These types of vaccines are currently in use for hepatitis B and are experimentally popular, being used to try to develop new vaccines against ebolavirus and human immunodeficiency virus (HIV) (Loomis & Johnson, 2015).

Toxoids (Anatoxin or Anatoxine)

Toxoids are usually bacterial exotoxins whose toxicity has been inactivated or suppressed either by chemical (formalin) or heat treatment, whereas other properties, typically immunogenicity, are maintained. Thus, when used during vaccination, an immune response is mounted, and immunological memory cells (T and B) are formed against the molecular markers of the toxoid without resulting in toxin-induced illness. There are toxoids for prevention of diphteria, tetanus, and botulism.

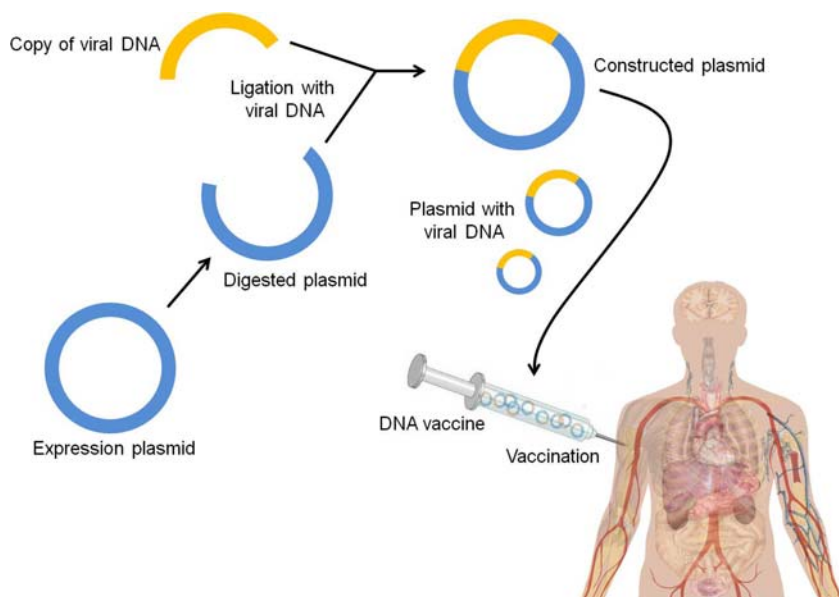


FIGURE 2.7 DNA vaccine technology.

Other Vaccine Technology

DNA Vaccine Technology

Plasmid DNA-encoding antigenic protein is injected directly into the muscle of recipients (Fig. 2.7). The resulting antigens are able to induce the correct immune response. In addition, after identifying the antigen, the route of the vaccine development is the same in all cases. DNA vaccines are used in case of many other diseases especially for HIV, malaria, and tuberculosis (Redwan, Matar, EL-Aziz, & Serour, 2008).

Recombinant Vaccine Technology

Recombinant vector vaccines are just like DNA vaccines, but they use attenuated virus or bacterium to introduce microbial DNA to cells of the body. Vector may be a bacterium or virus that is used as the carrier, which contain the components derived from microorganisms. The *Vaccinia* virus is the most commonly used vector in recombinant vaccine technology (Fig. 2.8) but also relevant to adenovirus and influenza virus (Arnon & Ben-Yedidia, 2003).

Adjuvants Vaccine Technology

Any substance which is able to enhance the immune response (cellular or humoral) to an antigen may be an adjuvant. It may be vitamins,

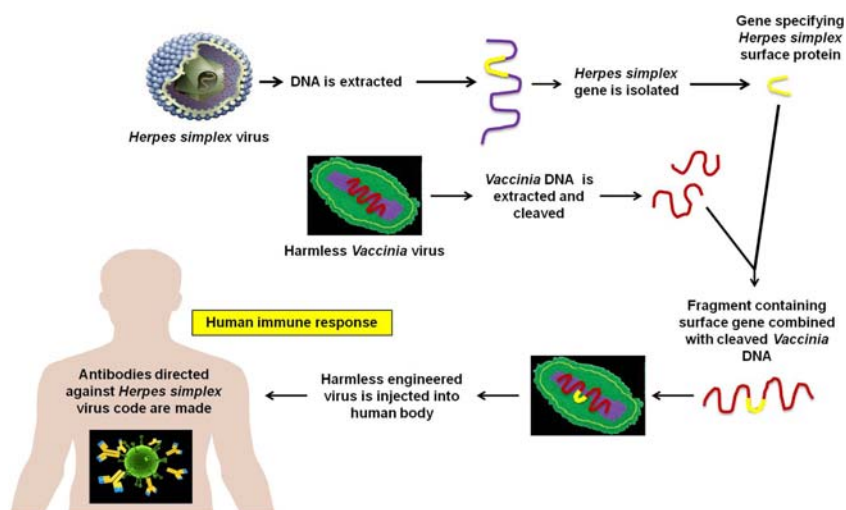


FIGURE 2.8 Recombinant vaccine technology.

phytohormones, bacterial compounds, or mineral salts. It is given in instances where the vaccine itself cannot induce sufficient response. By using adjuvants, the defense mechanisms may be activated earlier and last longer. Adjuvants can be combined with the vaccine either during its production or added to the vaccine just before it is administered in to body. In humans, most commonly used adjuvants are aluminum hydroxide, aluminum phosphate, or calcium phosphate hydroxide (Pingoud & Jeltsch, 2001).

BIOTECHNOLOGICAL TOOLS FOR INDUSTRIAL APPLICATION

Protein Engineering (PE)

In the last decade, genetic-engineering technology has been developed for cloning of gene encoding for any essential, specific protein found in nature. The pioneering gene cloning experiments of Herbert Boyer and Stanley Cohen in the early 1970s have been conducted in an era where proteins could be made and modified as never before. By precise manipulation of the appropriate regulatory signals, there may be possibilities to produce significant quantities of that protein in bacteria. Nowadays, artificial or chemical synthesis of DNA offers more prospects for developing PE technology to create novel proteins that are usually not found in nature.

PE is a complex and multidisciplinary field, where several different techniques and knowledge are applied in combination. The protein of interest needs to be first purified and characterized with regard to its functional properties, then to be cloned and expressed in a suitable host organism, and

subsequently to be modified so as to improve its performances. PE is different from genetic engineering in that the final product is modified in such a way that it does not reproduce so many of the concerns associated with genetic engineering for animal welfare. In this regard, engineered proteins more closely resemble to new chemical compounds from nonbiological sources, for which there is no any concern about safety and toxicity.

Strategies for Protein Engineering

Two strategies have come out to design proteins to work better under unusual conditions. The first employs SDM along with proteins structural information to rationally design new or improved function protein. More recently, this rational strategy has been attempted with some success to create functioning proteins de novo by insertion or nonsense mutation. In contrast to this, a nonrational approach called directed evolution employs RDTs to create thousands of possible variants and then uses high throughput screening methods such as DNA shuffling, family shuffling, or error-prone PCR (epPCR) either separately or in combination, to rapidly search for the one that offers the best solution. This strategy has emerged as a powerful alternative to rational methods particularly when relationship between structural and desired function is unclear, but where a representative screen can be developed. Direct evolution approach is more powerful than rational design.

Protein Engineering for Industrial Products

Protein Engineering for Industrial Enzymes

Since last many year biocatalysts have been successfully exploited by many food, chemicals, paper, and pharmaceutical industries due to their intrinsic ability to catalyze reactions with high catalytic efficiency and specificity under a variety of conditions. The increasing interest in applying enzymes in industrial and household catalysis has promoted the development of PE methodologies for novel biocatalysts with improved properties. Recent advances in RDT, genomics, and proteomics have promoted the development of new biocatalysts and biocatalytic processes. Since the beginning of large-scale recombinant enzyme production for industrial applications, PE has emerged as a powerful tool to improve enzymatic properties. Enzymes with the desired properties such as enhanced activity, high thermostability and specificity under industrial conditions can be obtained by optimizing process conditions and by PE (Table 2.7) (Singh, Tiwari, Singh, & Lee, 2013).

Protein Engineering for Biosensors

Any sensor incorporating biological or biomimetic material as the detector element is considered a biosensor. Biosensors capitalize on the specificity of biological recognition to provide tools for monitoring the presence of small

TABLE 2.7 List of the Enzymes Modified by Protein Engineering

Enzyme	Organism	Improved Property	Method	Application
Fructose biphosphate aldolase	<i>Escherichia coli</i>	Thermostability and organostability	DNA shuffling	Use in organic synthesis
Xylose isomerase	<i>Thermotoga neapolitana</i>	High activity on glucose at low temperature and low pH	RM	Used in preparation of high fructose syrup
Cyclodextrin Glucanotransferase	<i>Bacillus stearothermophilus</i>	Modulation of cyclizing activity and thermostability	SDM	Bread industry
Subtilase	<i>Bacillus</i> sp.	6-fold increase in caseinolytic activity at 15–25°C	DE and SDM	Detergent additives and food processing
Pyranose 2-oxidase	<i>Trametes multicolor</i>	Thermostability	SDM	Food industry
Endo- β -1,4-xylanase	<i>Bacillus subtilis</i>	Acid stability	RPE	Degradation of hemicelluloses
Endo-1,4- β -xylanase II	<i>Trichoderma reesei</i>	Increased alkali stability	SDM	Sulfate pulp bleaching
Endoglucanase	<i>Thermoascus aurantiacus</i>	4-fold increase in kcat and 2.5-fold in hydrolytic activity	SDM	Bioethanol production
Endoglucanase Cel8A	<i>Clostridium thermocellum</i>	Thermostability	SDM	Conversion of cellulosic biomass to biofuels
α -Amylase	<i>Bacillus</i> sp.	Thermostability	DE	Baking industry
	<i>Bacillus</i> sp. US149	Thermostability	SDM	Degradation of hemicelluloses
	<i>Bacillus licheniformis</i>	Acid stability	DE	Starch hydrolysis
(Continued)				

TABLE 2.7 (Continued)

Enzyme	Organism	Improved Property	Method	Application
Tagatose-1,6-Bisphosphate Aldolase	<i>Escherichia coli</i>	80-fold improvement in kcat/km and 100-fold change in stereospecificity	DNA shuffling	Efficient syntheses of complex stereoisomeric products
Prolidase	<i>Pyrococcus horikoshii</i>	Thermostability	RM	Detoxification of organophosphorus nerve agents
Alkaline amylase	<i>Alkalimonas amylolytica</i>	Oxidative stability	SDM	Detergent and textile industries
Glycerol dehydratase	<i>Klebsiella pneumonia</i>	2-fold pH stability; enhanced specific activity	RPE	Synthesis of 1,3-Propanediol
Laccase	<i>Bacillus HR03</i>	3-fold improved kcat and thermostability	DM	Catalyze oxidation of polyphenols, and polyamines
	<i>Pycnoporus cinnabarinus</i>	8000-fold increase in kcat/km	DE	Lignocellulose biorefineries, organic synthesis, and bioelectrocatalysis
D-Glucose 1-dehydrogenase isozymes	<i>Bacillus megaterium</i>	Thermostability	SDM	Measurements of blood glucose level
Galactose oxidase	<i>Fusarium graminearum</i>	3.4–4.4 fold greater Vmax/km and increased specificity	Error-prone PCR and screening	Derivatization of guar gum
Fructosyl peptide oxidase	<i>Coniochaeta</i> sp.	79.8-fold enhanced thermostability	DE and SDM	Clinical diagnosis
Cholesterol oxidase	<i>Brevibacterium</i> sp.	Thermostability and enzymatic activity	SDM	Detection and conversion of cholesterol

Feruloyl esterase	<i>Aspergillus niger</i>	Increase in half-life from 15 to >4000 min	DE and SDM	Degradation of lignocelluloses
Tyrosine phenol-lyase	<i>Symbiobacterium toebi</i>	Thermostability	DE	Industrial production of l-tyrosine and its derivatives
Protease BYA	<i>Bacillus</i> sp. Y	Specific activity1.5-fold higher	SDM	Detergents products
Superoxide dismutase	<i>Potentilla atrosanguinea</i>	Thermostability	SDM	Scavenging of O ₂ ^{•-}
Phospholipase D	<i>Streptomyces</i> sp.	Thermostability	SDM	Phosphatidylinositol synthesis
L-Asparaginase	<i>Erwinia carotovora</i>	Thermostability	DE	Therapeutic agent
Lipase	<i>Bacillus pumilus</i>	Thermostability and 4-fold increase in kcat	SDM	Chemical, food, leather and detergent industries
	<i>Geobacillus</i> sp. NTU 03	79.4-fold increment in activity, 6.3–79-fold enhanced thermostability	epPCR and SDM	Trans-esterification
	<i>Pseudomonas aeruginosa</i>	2-fold increase in amidase activity	RM and screening	Understanding lipase inability to hydrolyze amides
	<i>Candida antarctica</i>	20-fold increase in half-life at 70°C	Error prone PCR	Resolution and desymmetrization of compound
	<i>Candida antarctica</i>	Thermostability	MD and SDM	Detergent industries
β-Glucosidase	<i>Trichoderma reesei</i>	Enhanced kcat/km and kcat values by 5.3- and 6.9-fold	SDM	Hydrolysis of cellobiose and cellodextrins
Cellobiose phosphorylase	<i>Clostridium thermocellum</i>	Thermostability	RPE	Phosphorolysis of cellobiose
(Continued)				

TABLE 2.7 (Continued)

Enzyme	Organism	Improved Property	Method	Application
Phytase	<i>Penicilium</i> sp.	Thermostability	DE	Feed additives
Cot A laccase	<i>Bacillus subtilis</i>	120-fold more specific for ABTS	DE	Catalyze oxidation of polyphenols
β -Glucosidase	<i>Thermobifida fusca</i>	Thermostability	Family shuffling, SDM	Bioconversion of cellulosic biomass
Xylanase XT6	<i>Geobacillus stearothermophilus</i>	52-fold enhancement in thermostability, k_{opt} increase by 10°C, catalytic efficiency increase by 90%	DE and SDM	Bio-bleaching, Degradation of hemicelluloses
<i>p</i> -Hydroxybenzoate hydroxylase	<i>Pseudomonas fluorescens</i> NBRC 14160	Activity, reaction specificity, and thermostability	Combinatorial mutagenesis	Degrading various aromatic compounds in the environment

SDM, site-directed mutagenesis; RPE, rational protein engineering; RM, random mutagenesis, MD, molecular dynamics (Singh et al., 2013)

components in complex mixtures. The range of biological materials utilized in sensing schemes includes whole cells, DNA, RNA, protein, receptors, antibodies, enzymes, and biomimetic materials such as imprinted polymers (Dzyadevych et al., 2008).

Proteins are most used molecular recognition elements in biosensing technologies because of ligand diversity and transduction mechanisms. Glucose biosensor depletes reagents and requires a new sensor for each measurement. Most enzyme-based sensors are dependent on the external replacement of reagents or depletion of their analytes. A reagentless, nondepleting sensor would make continuous monitoring with a single sensor. The use of more generic observable properties will permit sensors for different analytes to be exchanged while using the same instrumentation.

Protein Engineering for Detergents

Proteases, lipases, amylases, and cellulases are most commonly used enzymes in textile industry to remove microbial reservoirs during washing process. These enzymes are isolated and screened for their hydrolytic activity on protein, lipid, and carbohydrates and used for other industrial applications. On the basis of hydrolytic reactions enzymes are classified into six catalytic groups such as serine, threonine, cysteine, aspartic, glutamic, and metallo-proteases (Rawlings, Barrett, & Bateman, 2011). Approximately, 40% of the total enzyme sales account for proteases.

Nowadays, highly stable and thermo resistant proteases are prepared by using PE technologies by modification of nucleophilic characters of these proteases, for example, subtilisin, is a nonspecific protease initially obtained from *Bacillus subtilis*. Subtilisin initiates the nucleophilic attack on the peptide (amide) bond through a serine residue at the active site (Jakob, 2013).

Protein Engineering for Drug Development

The early success of PE relies heavily on illumination of the biological possesses for drug development from plant and microbial sources. The first commercial application of PE for drug development was realized in the microbial production of human insulin. Some other drugs are listed in Table 2.8 that are produced by using PE technologies.

Metabolic Engineering (ME)

Metabolic engineering (ME) involves the modification of biosynthetic pathways to generate or improve novel biochemical products or phenotypes. Metabolic engineering offers production of safe and efficacious industrial products with high yield. An overview of ME has shown in Fig. 2.9, and it depends on many features for engineering of either specific product or whole metabolic pathway: (1) an appropriate microbial host, (2) metabolic route

TABLE 2.8 List of Drugs Produced Through Protein Engineering

Name of Drug	Active Molecule	Treatment for Disease
Activase	Tissue plasminogen activator	For dissolving blood clots
	Adenosine deaminase	Severe combined immunodeficiency
Aldurazyme	α -L-Iduronidase	Mucopolysaccharidosis type I
Avonex, Rebif, Betaseron	Interferon	Hepatitis B
Ceredase	Glucocerebrosidase	Type 1 Gaucher's disease
Engerix B	Hepatitis B surface antigen	Hepatitis B
	C1 inhibitor	Hereditary angioedema
Eprex, Epogen	Erythropoietin	Anemia
Factor IX	Factor IX	Hemophilia B
Factor VIII	Factor VIII	Hemophilia A
Genotropin, Humatrop, Serostin	Human growth hormone	Growth hormone deficiency
Humulin R, Actrapid HM	Insulin	Diabetes
Naglazyme	N-Acetylgalactosamine-4-sulfatase	Mucopolysaccharidosis type VI (Maroteaux–Lamy syndrome)
Neupogen	Granulocyte colony-stimulating factor	Cerebral ischemia
Pergonal	Follicle-stimulating hormone	Fertility issues in women
Pulmozyme	Dornase alfa	Cystic fibrosis

and corresponding gene that encode desired product, (3) strong and responsive genetic control system for selection of host and desired products, (4) proofreading methods for finding and resolving defects or error during engineering, and (5) cost and availability of substrate or starting materials.

Metabolic Engineering of Industrial Products

ME Approaches for Biofuel Synthesis

The desired biosynthetic pathways for advanced biofuels often require multiple enzymatic steps. Current engineering techniques are effectively altering enzymes or metabolic pathways to increase the flux toward biofuel synthesis.

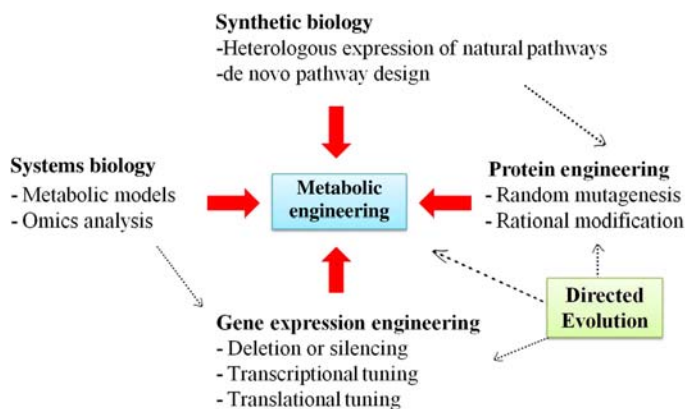


FIGURE 2.9 Overview of metabolic engineering process.

Common engineering strategies includes choice of plasmids and its copy numbers, promoter engineering, codon optimization of key enzymes, improvement of ribosome binding sites and knockout/knockdown of competitive pathways.

The promise of cheap biofuels production has yet to be fulfilled by using fermentation technology. Modern fermentation or ME is employing for development of biofuels with high economical values. ME depend upon various approaches such as: (1) development of a suitable biofuel developer with efficient metabolism, (2) carbon fixation due to limitations of ATP/NAD(P)H levels in engineered host, and (3) engineering of bioreactors/fermentors to control various parameters such as reactor geometry, aeration rate, oxygen/nutrient supply, P/O ratio, mixing quality, impeller selection, and operation cost to prevent the over secretion.

ME for Alcohols Production

Limitation of fossil fuels is a major issue of various biofuels producing industries, so biofuels production from renewable energy sources such as wood, crop waste, garbage, and microbial waste is become a focusing point of current research. Through the fermentation engineering ethanol is the higher produced biofuel than other alcohols, but due to hygrophobicity, high density, and vapor pressure, it is not good biofuel source. In comparison to ethanol, higher alcohols such as propanol, 1-butanol, isobutanol, 2-methyl-1-butanol, and 3-methyl-1-butanol have good biofuel properties similar to petroleum-based fuel. Though naturally microorganism does not produce these alcohols at high efficiency, ME has been applied to increase their rate of production. Some bacteria (*Escherichia coli*, *Zymomonas mobilis*, *Clostridium acetobutylicum*, *Synechococcus elongates*) and yeast (*Saccharomyces cerevisiae*, *Saccharomyces carlsbergensesiae*, *S. saki*,

Saccharomyces oviformis, *Candida utilis*, etc.) are engineered for production of higher alcohols by engineering of their fatty acids or amino acids pathway (Choi and Lee, 2013).

ME for Oils Production

Over the last century the world has become dependent on fossil fuels as the primary source of hydrocarbons for production of energy and industrial products. These resources are finite and nonrenewable and their exploitation releases massive amounts of CO₂ into the atmosphere. High CO₂ concentration becomes a major environmental issue. So identification of sustainable replacement by ME of plants and microorganism has been an ongoing area of research for many developing countries including India. Plant triacylglycerol oils have shown considerable potential to replace petrochemicals in a wide variety of applications. Vegetable oil-based lubricants have biodegradability, efficient lubricity, good oxidative stability, and can control viscosity at lower temperatures to prevent hydrolytic cleavage of ester bonds. The vegetable oil industry has, therefore, focused on producing oil for manufacture of industrial products.

ME for Drugs Production

Production of pharmaceuticals by modification of microorganism is an ongoing area of research. Genes or their parts have been transferred from one microorganism in to other (heterologous host) by expressing the biosynthetic pathways are an attracting way of increasing pharmaceutical yield (Zadran & Levine, 2013). Heterologous host use cheaper feedstock, easy to grow and has well-designed genetic tools for efficient ME approaches to achieve higher production. Thus, production of chemicals compounds in heterologous hosts provides an alternative route for drug development. Till now, various microorganism has been engineered for production of pharmaceutical products (Table 2.9).

CONCLUSION

One of the major challenges for the 21st century is to develop a sustainable bio-based financial system that uses environment-friendly bioprocesses and renewable bioresources. Biotechnological tools are used for adapting and modifying the biological organisms, products, processes, and systems present in our environment. They are also used in industries for cost reduction and improvement of environmental performance to complement the conventional chemical technologies. Improved knowledge of biodiversity, biology, ecology, and biotechnology makes it feasible, both sustainable increase of biomass productivity in agriculture and efficient utilization of biomass in sustainable manner. These tools are expected to play a major role to improve the global food availability at reasonable prices for the rural poor in most parts of the world. GE crops, having properties such as resistance to biotic and abiotic stress factors, are useful for small

TABLE 2.9 Some Engineered Hosts for Production of Pharmaceuticals

Engineered Host	Pharmaceuticals (Drug)
<i>Amycolatopsis mediterranei</i>	Rifamycin
<i>Aspergillus nidulans</i>	Asperfuranone, monodictyphenone
<i>Candida albicans</i>	Dihydrofarnesol
<i>E. coli</i>	Piceatannol, cinnamycin, 6-deoxy erythronolide B, theophylline, amorphadiene, taxadiene, glucaric acid, fatty acids
<i>Nicotiana benthamiana</i>	Diterpenes
<i>Pantoea agglomerans</i>	Andrimid derivatives
<i>Pleosporales</i>	Phomoidrides
<i>Pseudomonas putida</i>	Myxochromide S
<i>S. cerevisiae</i>	Parahydroxybenzoic acid, miltiradien, benzyloquinoline alkaloids, morphinan, valencene
<i>Streptomyces cinnamonensis</i>	Polyketide derivatives
<i>Streptomyces coelicolor</i>	Epothilone

scale farming systems and can easily be included without adjusting traditional cropping pattern. The comparatively low net cost for adopting biotechnological tools at the farm level also makes it valuable for semisubsistence agriculture. The application of these tools across various industries has consistently led to both economic and environmental benefits resulting in to less expensive processing, entirely new products, enhanced product quality and eco-friendly processing relative to conventional operations.

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Chapter 3

Sustainable Agriculture and Food Security

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INTRODUCTION

As the global population continues to grow exponentially, much more effort will be needed in order to sustainably increase agricultural practices, improving the global food demands, limits food losses and waste, and ensure that all who are suffering from hunger and malnutrition have right to use nutritious food. Agriculture system must become more productive and less wasteful worldwide. Sustainable agricultural practices including both production and consumption must be adopted from a holistic and integrated approach. Traditional farmer’s knowledge about productive food systems have been

enriched by the latest scientific knowledge, which support through sound and sustainable, water, land, soil, nutrient and pest control, and the more wide use of organic farming. The Food and Agriculture Organization (FAO) defines food security as “a situation when all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life” (FAO, 1996). By the year 2030, the global population is expected to reach 8.5 billion, which will be 34% increase in today’s population. To keep the pace, it is essential to increase the annual meat production by over 200 million tonnes to reach 470 million tonnes and the annual cereal production will need to rise to about 3 billion tonnes from 2.1 billion tonnes today. This will surely mark the worst economic crisis in the country, which makes the people to move around begging with bowls to ensure food supply for survival. This makes us to look into various factors affecting the crop production and modify our strategy to address these problems, with the optimum use of the available technologies and resources to reach the new target.

Farmers, particularly the small holders, need proper orientation to take suitable decisions on crop selection, investment in various inputs, storage and marketing, based on the information on technical developments, government policies, and prices of inputs and outputs. Large and elite landholders should also be encouraged to adapt these ecofriendly measures for profitability and environmental safety. Considering the present levels of crop yields in other countries, the task is within the reach of the Indian farmers. In India, the average cereal crop yield is 1935 kg/ha, as compared to China which is 4329 kg/ha, in the United States 4040 and 2757 kg/ha in the world. India does not lag behind significantly with regard to per capita land holding and percentage of the crop lands covered under irrigation. With respect to the use of fertilizers, India is significantly lower when compared to China, but closer to North America.

The crop-wise comparative data on yield, cropping area, and production in 1980–81 and 1994–95 indicate that the application of modern agricultural production technologies in the past to boost the yields were limited to only a few crops such as maize, wheat, and paddy. It is because of only an increase in the yield of wheat that the average yield of cereals has increased in recent years. Other than wheat, no crops have exceeded this average yield, although the possible yield of these crops is in the range of 2500 and 3500 kg/ha. The major crop is rice with over 43 million ha, contributing about 42% of the total food grains produced in the country. Application of higher doses of chemical fertilizers has a direct influence on the crop yields. In China, the cereal crop yield is 4329 kg/ha, the highest in the world, the average fertilizers applied for cereal crops is 284 kg/ha, whereas the world average is 96 kg/ha. However in Europe, the average cereal crop yields is 4295 kg/ha, only 1.0% lower as compared to China with over 30% lower doses of fertilizers. The average cereal yield in the United States is

4040 kg/ha, with only 87 kg fertilizers per hectare. The reduction in the crop yield by 6.7% with over 70% reduction in fertilizer application highlights the scope for careful use of fertilizer for sustainable crop production.

Increase in food production in the country does not automatically ensure food security, if the poor do not have the power to buy. Therefore, participation of small farmers in food production is required to achieve food security in the country. Most of farmers are being illiterate and having failed earlier either in adapting new technologies or repaying the loan provided under various schemes sponsored by the government, they have lost hopes both in themselves and the extension agencies. They not only need support to procure inputs but also to gain confidence. Agriculture and other natural resource-based enterprises are the foundation for economic growth in many developing countries. With no significant room to expand areas of cultivation, good farming practices and stewardship of the available land are necessary to increase agricultural productivity, ensure economic growth, protect biodiversity, maintain sufficient amounts of clean water, and meet the increasing food demands of a growing global population.

With initial support to develop their skills, capabilities, and resources, small farmers can take their own decisions on use of external inputs and cropping pattern to enhance crop production. Simultaneously, the large holders can be encouraged to make necessary investments on external inputs with advance technological support to maximize the production. As the small farmers generally follow the rich and progressive farmers, in this process of capacity building, both poor and rich can contribute their best to increase the food production.

Replacement of local seeds with change of crops, certified seeds, and use of mineral nutrients, improved farm implements, installation of irrigation system, and plant protection measures are some of the new initiatives to enhance the crop yields. Marketing, postharvest processing and storage are other areas where both large and small holders have been incurring heavy losses. This area needs infrastructural development and introduction of modern technologies which can add value to the product and reduce the cost of handling.

FAO (1988) has defined sustainable agricultural development as “the management and conservation of the natural resource base, and the orientation of technological change in such a manner as to ensure the attainment of continued satisfaction of human needs for present and future generations. Sustainable agriculture conserves land, water, and plant and animal genetic resources, and is environmentally non-degrading, technically appropriate, economically viable and socially acceptable.” According to vonWiren-Lehr (2001), implementing and assessing sustainability in agriculture can be undertaken by using goal-oriented strategy approaches. The goal of sustainable development is to end hunger, achieve food security, and improved nutrition. Empowering small farmers, promoting gender equality, ending rural poverty, ensuring healthy lifestyles, and tackling climate change are some of the inter

linkages that supports sustainable agriculture. Beyond adequate calories intake, proper nutrition has other dimensions that deserve attention, including micronutrient availability and healthy diets. Inadequate micronutrient intake of mothers and infants can have long-term developmental impacts. Unhealthy diets and lifestyles are closely linked to the growing incidence of noncommunicable diseases in both developed and developing countries. Extreme poverty and hunger are predominantly rural, with smallholder farmers and their families making up a very significant proportion of the poor and hungry. Thus, eradicating poverty and hunger are integrally linked to boosting food production, agricultural productivity, and rural incomes.

Agriculture systems worldwide must become more productive and less wasteful. Sustainable agricultural practices and food systems, including both production and consumption, must be pursued from a holistic and integrated perspective. Land, healthy soils, water, and plant genetic resources are key inputs into food production, and their growing scarcity in many parts of the world makes it imperative to use and manage them sustainably. Boosting yields on existing agricultural lands, including restoration of degraded lands, through sustainable agricultural practices would also relieve pressure to clear forests for agricultural production. Wise management of scarce water through improved irrigation and storage technologies, combined with development of new drought-resistant crop varieties, can contribute to sustaining dry land's productivity. Halting and reversing land degradation will also be critical to meeting future food needs.

Since the mid-1990s, the number of malnutrition has increased, despite the fact that the farmers have been producing an average of 4600 kcal per person, or about twice as much as needed globally. This imbalance may include not only the losses between harvest and consumption, an increasing amount of agricultural land devoted to the production of biofuels, but also the consumption behavior. At the same time, ecosystems are more and more degraded, natural resources are shrinking, and the impacts of climate change are painfully felt in many parts of the world, especially to those already living in below poverty. To ensure sustainable agriculture and food security, following targets has to be set:

1. End malnutrition and hunger, including over nutrition, under nutrition, and nutritional deficiency, so that all people enjoy the right to have food at all time.
2. Ensure that all small farmers and other rural communities enjoy a respectable livelihood and income, and safeguard their right to access to productive resources.
3. Achieve the transformation to diverse, sustainable and resilient agriculture and food systems that conserve natural resources and ecosystems and realize a land degradation neutral world.
4. Accomplish zero postharvest and other food losses and waste.

5. Establish transparent, inclusive, and equitable legislative and other decision-making processes on food, nutrition, and agriculture themes at all levels.

Transfer of Technologies

The modern agricultural practices have been currently adapted for a few selected crops. These include the use of biofertilizers, efficient irrigation systems, biopesticides, improved seeds, and safe agrochemicals, which have direct effect on crop yields and cost of production. Simple techniques for water, soil, and plant analysis to maintain nutritional balance, pest and weed management to reduce nutrient losses are some areas where technology transfer are lagging behind. Farmers can be trained to make careful use of nutrients based on the soil fertility, soil moisture availability, organic matter content, and the crop requirement, as the balance between macro- and micro-nutrients, organic manure, is very critical to induce flowering and crop yields. It is, therefore, required to strengthen the linkage between the State Agricultural Extension Department and Agricultural Research Institutions with the farmers at the village level, through local voluntary agencies. Agriculture and other natural resource-based enterprises are the foundation for economic growth in many developing countries. A transition is needed toward an optimal and renewable use of nature and natural resources and toward sustainable production and processing systems. These systems will need to produce more food, fiber, and other bio-based products with reduced inputs, environmental impacts, and greenhouse gas (GHG) emissions and with enhanced ecosystem services, zero waste, and adequate societal value.

Information Support

There are many elements of traditional farmer knowledge that, enriched by the latest scientific knowledge, can support productive food systems through sound and sustainable soil, land, water, nutrient and pest management, and the more extensive use of organic fertilizers. An increase in integrated decision-making processes at national and regional levels are needed to achieve synergies and adequately address trade-offs among agriculture, water, energy, land, and climate change. There has never been a more urgent need to train food security researchers who are equipped with skills in agronomy, plant pathology, plant disease and plant genetics, and knowledge of modern agricultural systems and agricultural policies. Building resilience of local food systems will be critical to averting large-scale future shortages and to ensure food security and good nutrition for all. Sustainable agriculture integrates environmental health, economic viability, and social equity to ensure long-term productivity of natural resources and improved livelihoods. It helps

reduce the risks in the developing countries of complex problems like water variability and scarcity—important because agriculture constitutes approximately 70% of water consumption in the developing world, increasingly competing with demand for domestic, industrial, and ecosystem services.

Advice on suitable cropping, weather forecasts, information service on market demand, periodic reports on area under various crops, and scope for further expansion and incidence of diseases and pests, through mass media, can help the small and huge farmers to take suitable decisions. These support services can be made available to farmers regularly by the government or from farmers' organizations.

All nations are facing more difficulties in remaining competitive in the global market for many reasons. Some of them are the soil infertility and the huge use of expensive external nutrients, notably nitrogen and phosphorous, in which agriculture is dependent on imported products like fertilizers produced with expensive industrial processes, which generates GHGs. Therefore, sustainable crop management is needed to maintain and increase soil fertility. Improper soil and water management, and the overuse of chemical fertilizers in crop production systems, leads to economic loss for the farmer and an important burden to the environment and subsequent impact on human health, as they contribute to surface water and ground water pollution, GHGs emissions, the build-up in soil contaminants, such as heavy metals and organic pollutants. Soil management and optimization of water and fertilizers are of supreme importance for conciliating the long-term sustainability and the necessary competitiveness of the entire intensive crop production sectors. It can be achieved through the following:

1. Novel and effective strategies to improve the management of water and external nutrient inputs and optimize their effectiveness at field level to improve both quality and yield.
2. Evaluate real benefits that agronomic techniques and soil-improving cropping systems, e.g., crop rotations, exactitude farming, and conservation agriculture as well as to identify and minimize limitations and drawbacks.

Adapting sustainable crop management strategies can be achieved through the following:

1. Improvement of surface and ground water levels.
2. Reduction of field contaminations with heavy metals and toxic chemicals.
3. Bioconservation of wildlife and biodiversity.
4. Improvement of human health, by the reduced release of GHGs and pollutants.
5. Improvement of soil quality by reduction of soil erosion and maintain its structural integrity.
6. Maintaining limited soil disturbance by better exploitation of soil biodiversity.

ORGANIC FARMING FOR SUSTAINABLE AGRICULTURE

In the process of reducing or avoiding the use of external inputs, if the small farmers start using organic farming, the productivity of the soil can also be conserved and consumers can get healthy food. With better awareness of organic food and its benefits, the consumers will be ready to pay a good price for ecofriendly produce. Hence, sustainable agriculture can be attractive particularly for small farmers.

For most developing countries, agriculture remains the key sector for the economic progress. It is important for establishing food security, conserving the vital natural resources and alleviating poverty line, represent world's present and future generations for their well-being and survival. Bioorganic farming seems to be more appropriate for sustainability of natural resources and environment. It is a production system which includes maximum use of organic materials such as animal residue, legumes, crop residue, farm wastages, growth regulators, and biopesticides and discourages the use of chemically produced fertilizers, for maintaining soil fertility and productivity, and pest control under conditions of sustainable natural resources and healthy environment. This form of farming can also be called sustainable farming or sustainable agriculture. The principles of this method are as follows:

1. Maintain soil fertility for optimum production relying on renewable resources.
2. Use and develop appropriate techniques based on biological systems.
3. Aspire for optimum nutritionally enriched food.
4. Organize the production of crops and livestock and the management of farm resources so that they complement rather than conflict with natural system.

Farmers produce more better-quality food and achieve higher income by organic farming. Organic farming is based on a combination of indigenous knowledge and traditional and modern agriculture research. In traditional agriculture systems, organic methods often enable a high yield in production. The use of chemical fertilizers in modern farming leads to adverse effect on water, soil, food, and atmospheric environment, in developed and developing countries. Throughout the organic literature, conventional systems differs from organic farming with respect to their effects on physical properties of soil, nutrient flow within the soil, nutritional value of the harvested crop, and crop health.

Important components of organic farming are as follows:

1. Soil and crop management
2. Agriculture waste recycling
3. Biological weed management
4. Industrial and domestic waste recycling
5. Energy reuse
6. Food quality improvement

7. Eco-agriculture
8. Integrated intensive farming system

Benefits of Soil Organic Matter

Soil organic matter acts as “cement” for holding silt and clay particles together, contributing to structure of the soil, increasing water holding capacity and control of soil erosion. It helps in holding micronutrients in the soil, otherwise which might be washed out from the soil surface. The organic substituent in the humus layer may act as plant growth enhancers. It acts as a storage house for slow release of elements like nitrogen, phosphorus, and sulfur for plant and microbial growth.

Seed Treatments for Sustainable Agriculture

Seed is a basic input for sustained growth in agricultural productivity. The seed-borne and early-season diseases and insects create major hurdle, if not managed at proper time. Emphasis on present-day agriculture is to give more productivity by utilizing lesser land, water, and manpower. Since time immemorial, the ecofriendly disease-management practices like sanitation, mixed cropping, crop rotation, fallowing, adjustment of date of sowing, summer plowing, composting, green manuring, etc. to combat with phytopathogens have already lost their ability and has been reevaluated.

Seed treatment has some advantages over other crop management measures such as:

1. Reduction in initial inoculums
2. Increase seed vigor which is the key of successful field emergence and establishment
3. Even and uniform application of the chemical
4. More alternatives available to chemical in effective manner
5. It lessens the environmental side effects, viz. reducing risk to nontarget organism
6. Breaking of seed dormancy and improve emergence and plant stand
7. Combination of treatment can be applied more precisely

Biofertilizer as a Potent Nontraditional Additive for Organic Farming

Biofertilizer is a substance which contains microorganisms, when applied to the field, colonizes the plant rhizosphere or enters into the plant vascular system and promotes growth, by increasing the micro nutrients availability to the plant. Chemical fertilizers directly increase soil fertility by adding nutrients. Biofertilizers add nutrients through the natural processes by fixing atmospheric nitrogen and stimulating plant growth through the synthesis of growth promoting elements. Based on their nature and function, they can be grouped in different ways.

Types of Biofertilizers

Chemical fertilizers are being used in higher amounts in order to increase the crop yield. However, chemical fertilizers above threshold level pollute the water bodies, besides getting stored in crop plants. This has made the environmentalists to switch over to organic farming. Organic farming is the production of unpolluted crops through the use of biofertilizers and biopesticides which provide optimum nutrients availability to plants, keeping pathogens and pests in control.

1. Nitrogen fixing free living bacteria: e.g., *Azotobacter*, *Clostridium*, *Bacillus polymyxa*, *Beijerinckia*
2. Nitrogen fixing free living Cyanobacteria: e.g., *Nostoc*, *Anabaena*, *Aulosira*, *Totipotrix*
3. Partial association of nitrogen fixing bacteria: e.g., *Azospirillum*
4. Symbiotic nitrogen fixing bacteria: e.g., *Actinomyce*, *Rhizobium*, *Ardisia*
5. Symbiotic nitrogen fixing cyanobacteria: e.g., Blue-green algae
6. Microphos biofertilizers: e.g., *Aspergillus* species, *Pseudomonas striata*
7. Plant growth promoting rhizobacteria: e.g., *Pseudomonas fluorescens*
8. Mycorrhiza: Ectomycorrhiza, Endomycorrhiza

Recommended use of biofertilizers for different crops, which include *Rhizobium* (pulses, chickpea, groundnut, soybean, beans, lentil, lucern, berseem, greengram, blackgram, cowpea, and pigeonpea), *Azotobacter* (cereals, wheat, oat, barley, mustard, seasmum, linseeds, sunflower, castor, and millets), and *Azospirillum* (rice, maize, and sorghum) by either seed or soil treatment.

Importance of Biofertilizers

The following points can be considered for the efficient biofertilizers:

It should avoid pathogen growth, improve soil texture, produce vitamins and help in plant growth promotion, increase the crop yield, environment friendly, solubilize phosphorus and helps in uptake to the plants, suppress soil borne diseases, economical, and reduce the consumption of chemical fertilizers.

FOOD SECURITY AND CLIMATE CHANGE

In the 21st century, food security is one of the major challenges with respect to climate variations. Adverse climate change affects cereal production, through water and heat stress, but is also associated with disease and pest dynamics, water logging, and frost. Climate change is a repeated force of livelihood change, as increases in unpredictable and unusual weather patterns affect communities globally. Livestock holders from nomadic and settled communities with varied herd compositions must be included in the intensification strategies regarding conversation about climate change on food

security. Even without any alteration for climate changes, increasing populations, losing ground water supplies, changing diets, urbanization, and the additional demand on cereals like maize for fodder and fuel poses significant challenges for cereal production in near future (Hubert, Delrieu-Trottin, Irisson, Meyer, & Planes, 2010). The demand for cereals globally is expected to reach 3 billion tonnes by 2050 (Alexandratos & Bruinsma, 2012). Various qualitative indicators of heat stress are reduced seed set, shriveled seed, reduced grain size, quality, pollen sterility and stigma drying (for wheat), nonviable pollen, poor anther dehiscence, poor pollen growth, reduced pollen deposition on stigma and early embryo abortion (for rice), leaf firing, tassel blast, pollen sterility, accelerated senescence, barren plants and reduced seed set (for maize), reduced seed set, pollen sterility and stigma drying (for pearl millet) and reduced seed set, and pollen sterility and ovule sterility (for sorghum). In most crops, drought and heat stress tend to be stronger as the crop cycle progresses. This is especially the case for drought because (1) in most rain fed environments, precipitation tends to reduce as the cycle progresses; (2) stored soil moisture becomes increasingly depleted over time; and (3) increasing temperature increase vapor pressure deficit and therefore crop evaporative demand for water (Reynolds et al., 2016).

Some of the qualitative indicators for drought stress, for wheat are, early flowering and poor grain filling; for rice, poor panicle emergence, decline in leaf expansion, rate, early senescence, inferior grain set, and reduced grain weight; for maize, leaf senescence, increase in anthesis-silking interval, barren plants, reduced grain set, and poor tip filling of the ears; for pearl millet reduced seed size and seed set, poor tillering; for sorghum, inhibition of panicle exertion, decreased seed set, poor grain filling, and smaller grain size.

According to UN (1975), food security was defined as the “availability at all times of adequate world food supplies of basic foodstuffs to sustain a steady expansion of food consumption and to offset fluctuations in production and prices.”

Four Dimensions of Food Security

Food security exists when all people, at all times, have physical and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life. Four dimensions of food security include (1) physical availability of food, (2) economic and physical access to food, (3) food utilization, and (4) stability of the other three dimensions over time.

The Duration of Food Insecurity

Food security analysts have defined two general types of food. They are (1) chronic food insecurity which is long term or persistent occurs when people are unable to meet their minimum food requirements over a sustained period

of time that results from extended periods of poverty, lack of assets, and inadequate access to productive or financial resources and it can be overcome with long-term development measures such as education or access to productive resources, (2) transitory food insecurity is a short-term and temporary occurrence when there is a sudden drop in the ability to produce or access enough food that results from short-term shocks and fluctuations in food availability and food access, food prices, and household incomes, and it can be overcome with early warning capacity and safety net programs.

The Severity of Food Insecurity

When analyzing food insecurity, it is not only enough to know the duration of the problem that people are experiencing but also how severe or intense the identified problem is on the total food security and nutritional status. This knowledge will influence the nature, extent, and urgency of the assistance needed by severely affected population communities. Food security analysts developed different “scales” or “phases” to “grade” or “classify” food security which have been developed by using different indicators and cut-off points or “benchmarks.”

The integrated food security phase classification (IPC) is a classification system for food security crises based on a range of livelihood needs. Different levels of IPC classification and their indicators which include generally food secure (crude mortality rate), chronically food insecure (malnutrition practice), acute food and livelihood crisis (food access/availability), humanitarian emergency (water access/availability), and famine/humanitarian catastrophe (coping strategies, livelihood assets).

Vulnerability

The vibrant nature of food security is implied when we consider about people who are experiencing food insecurity and prone to more vulnerable in near future.

Vulnerability is defined in terms based on three critical parameters:

1. Outcome
2. Variety of risk factors
3. Inability to manage risks

Certainly a person can be vulnerable to hunger at a given point in time. Vulnerability analysis suggests two main intervention options:

1. Decreasing the degree of exposure to the hazard
2. Increasing the survival

Based on vulnerability point of view, food security programs and policies strengthen their efforts from addressing current constraints to food consumption which include actions and also address the future problems to food security.

Hunger, Malnutrition, and Poverty

Hunger is usually understood as a painful sensation or uncomfortableness caused by insufficient food energy sources. Scientifically, hunger is referred to as food scarcity. Simply, all hungry persons are food insecure, but not vice versa as there are other reasons of food insecurity, including those due to lack of micronutrients. Malnutrition results from deficiencies, imbalances, or excesses in the consumption of macro- and/or micronutrients. Malnutrition may be a possible outcome of food insecurity, or it may relate to nonfood factors, such as:

- inadequate care practices
- lack of proper health guidelines
- unhealthy environment.

Although poverty is unquestionably a cause of hunger, lack of proper nutrition is an underlying cause of poverty. Widely used definition of poverty is: “Poverty encompasses different dimensions of deprivation that relate to human capabilities including consumption and food security, health, education, rights, voice, security, dignity, and decent work.”

One of the major challenges is how to secure and provide healthy and nutritious food for exponentially growing populations in an ecofriendly and suitable manner. Inadequate nutrition for infants leads to irreversible brain development which contributes to death. This may result not only in poor health in adulthood but also early death, which is a major backdrop for economic and social development of countries (Black et al., 2013; Hoddinott, Alderman, Behrman, Haddad, & Horton, 2013). Approximately, estimated 160 million stunting children below 5 years of age reduced to 11% from 26% since 1990 (Black et al., 2013), but still 50 million children ahead for nutritious food (WHO, 2003). Worldwide, about 2.1 billion people are suffering from overweight and obesity; among them 41 million children who are below 5 years of age are overweight and 2/3 of children reside in low and middle income countries (Black et al., 2013; WHO, 2003). It threatens global health systems which are linked to noncommunicable threats such as cancer, diabetes, and cardiovascular diseases. Food insecurity, malnutrition, and poverty are deeply interrelated phenomena

Chronic Food Insecurity: Lack of minimum requirement of food to the people for a sustained period of time due to extended periods of poverty, lack of assets, and inadequate access to productive or financial resources can be called as chronic food insecurity.

Acute or Transitory Food Insecurity: Sudden lack of food or reduction in the ability to produce or access minimum requirement of food due to short-term shocks and fluctuations in food availability and food access, including year-to-year variations in domestic food production, food prices, and household incomes can be defined as acute or transitory food insecurity.

Demand for Food Products

A food demand system relies on energy, variety, and tastes of foods. By specifying utility as an explicit function of these characteristics, the entire matrix of demand elasticities can be derived of foods and nonfoods from prior selection on elasticities, while avoiding any assumption of separability between different foods. The following framework can explain why poorest groups often are most price-responsive but also account for highest price-responsiveness by middle income groups.

- There is no fixed habit there may be a considerable measure of elasticity of demand because of the possibility of the substitution of one food item for another. But the consumption of one food item in place of another cannot materially increase or decrease the amount of food as a whole.
- The amount of food waste is another factor that must be considered in estimating the degree of elasticity of demand for a given food product
- According to recent government investigations, the waste in families in the United States with incomes of less than \$800 per annum amounts to <4%, whereas in the case of families with incomes between \$1000 and \$3000, the waste frequently amounts ranges 10% to 25%.

Household Food Security

A household is food secure when it has access to the food needed for a healthy life for all its members (adequate in terms of quality, quantity, and safety and culturally acceptable) and when it is not at undue risk of losing such access. Food security at global or national level may not usually address the household level food security problem. The relationship between national food security and household food security is less prominent in the developing countries than in developed ones. Therefore, specific policies are required to address household-level food insecurity, and these policies should be contextual and problem specific.

Characteristics of Household With Very Low Food Security

- Members of household (mainly adult) worried that their food would run out before they got money to buy more.
- Food they bought just didn't last and they didn't have money to get more.
- They couldn't afford to eat balanced meals have to rely on inexpensive nonnutritious food.
- An adult had to cut the size of meals or skipped meals because there was not enough money for food.
- They had to eaten less than they felt they should because there was not enough money for food.

- They had been hungry but did not eat because they could not afford enough food.
- They had to acquire food through socially unacceptable means such as charitable assistance, buying food on credit, etc.

Similarly *Community Food Security* (CFS) has been defined as follows: CFS exists when all citizens obtain a safe, personally acceptable, and nutritious diet through a sustainable food system that maximizes healthy choices, community self-reliance and equal access for everyone. CFS is a relatively new food security-promoting strategy that considers all the factors within a region or community's food system that influence the availability, cost, and quality of food to households, particularly those in lower income communities. CFS is as much an antihunger as it is a community development strategy. It addresses multiple needs and problems within a local food system. CFS is both a goal and a method that embraces the full range of food chain activities—natural resources and agriculture, processing and distribution, nutrition and health, and public policy—and promotes a system's approach to food problems. Although the goal of CFS is the same as other approaches—to end hunger and food insecurity—its method is decidedly different. CFS, in its fullest expression, draws on a range of community food system resources, invites the participation of many individuals and sectors, and promotes solutions that reduce food insecurity and build the health and well-being of the wider community. Three other components of the CFS definition—sustainability, social justice, and democratic decision-making—need brief explanations. As CFS is concerned with the viability of the natural resource base that produces our food as well as the food system's current dependence on nonrenewable energy sources (e.g., fossil fuels), it promotes sustainable farming practices. Likewise, CFS supports strong marketing channels between consumers and farmers that are in the same region to decrease the distance that food must travel (food travels an average of 1500 mi before it reaches its final destination).

The nutritional status of each member of the household depends on several conditions being met: the food available to the household must be shared according to individual needs; the food must be of sufficient variety, quality, and safety; and each family member must have good health status in order to benefit from the food consumed. FAO recognizes that healthy, well-nourished people are both the outcome of successful social and economic development and constitute an essential input into the development process. Community-centered approaches for improving nutrition build capabilities and empower communities to effectively demand services and productive resources and at the same time support local initiatives for implementing food and nutrition programs. This involves increasing the participation of communities in the design, implementation, and monitoring of development programs and interventions. Achieving household food and nutrition security requires coordination among local institutions that can or should support food insecure groups. A key dimension of this strategy is enabling

households to maximize food security and nutrition with existing household resources, while also striving to increase such resources. This requires a process of effectively mobilizing communities and shifting from a centralized to a more decentralized approach, with wider participation on the part of the community. Community-centered nutrition programs aim at building capacities and empowering people to create a demand for their own household food security and nutrition improvement. This involves enlisting in them a strong sense of ownership for developmental programs, which in essence, become community investments for promoting their own nutritional well-being and development. A number of activities that address problems of household food insecurity and the various forms of malnutrition are being undertaken by FAO in both urban and rural areas. An important focus is community empowerment, with appropriate support from the various governmental levels and civil society institutions. At the community level, targeted and coordinated efforts focusing on improving household food security, fostering people's participation, and empowering women and marginal groups are needed to address local food and nutrition problems. Such efforts include: participatory appraisal and planning methods; expanding and diversifying food production and ensuring availability at the local market; improving food preservation and storage; improving water supplies; expanding and diversifying income-generating activities; providing nutrition education and training; and ensuring access to basic health care and care systems. In industrialized countries, income-related food security is measured at both the individual and household levels whereas in nonindustrialized countries it is most often measured in terms of under-nutrition and malnutrition through anthropometric measurements. Indicators of individual food insecurity include limited food selection, suboptimal nutrient intakes and severe nutrient inadequacies. Household food insecurity measures food intake of adults and children as a group within the home in relation to household income and food cost expenditure. Community programs have proven to be an effective way to achieve CFS by overcoming barriers to food security. Community programs can offer short and long-term approaches to CFS and can have a range of influence and effectiveness. For example, smaller short-term approaches include ensuring community members are aware of existing food assistance programs, social services, and job training workshops in their community. However, it is important to note that evidence is limited that such programs effectively address barriers such as inadequate income.

To achieve more long-lasting solutions, changes in the food system of a community may be necessary. Connecting social services with the food system will strengthen partnerships across sectors and help to build capacity among community members. For example, connecting dietitians with farmers to create programs such as community supported agriculture and place-based institutional procurement strategies can help both the health of the community and welfare of the farmers. Also a shift in the use of temporary solutions, such as food banks to community gardens, builds capacity among

community members by teaching skills in growing their own food and increases their self-reliance. Advocacy is another important strategy in achieving CFS. Promoting locally grown, seasonal, and organic foods in the community can help to support the local economy and protect the environment. Provision of opportunities and enabling conditions for more food to be produced and purchased locally can be done by promoting the benefits of local consumption to the public and by providing incentives and subsidies to farmers. Increasing food production in a community creates a more sustainable food system and lessens reliance on imports from other communities. Advocacy is also important for social equity to ensure that everyone in the community has access to nutritious foods and the ability to participate in decision-making. Conducting research in a community to determine the cost of a nutritious diet and the availability of healthy foods in low-income neighborhoods can result in data that can be used to advocate for policy change. Advocating for increases in minimum wage to a living wage and more affordable housing is thought to allow community members to have more money available to purchase food. For ethical concerns about where food comes from or if it was harvested in an environmentally sustainable way, one can advocate for stricter regulations on food labels.

From these definitions, achieving food security seems utopian (at least ideal) and no country could hope to reach in reality. Therefore, for specific program/project or particular nation definition of food security should be something achievable or measurable at least for certain duration. But, these definitions should cover the basics. No matter how, we define food security, having enough to eat regularly for active and healthy life is the most essential human need. Many developing countries, especially in South Asia and Africa, haven't been able to fulfill this vital need even today.

Foodshed

In 1929, "Foodshed" was defined for the first time, as the geographic area that represents the flow of foodstuffs from their origin to consumers, determined by economic principles. The concept derives from that of "watershed," as both are portions of a region where resources (food or water) are transferred to nurture the region itself. However, food shed boundaries are not limited only to geographic and spatial. The food shed brings together natural aspects (shed), cultural (food) and expressing the coexistence of society and nature, and interacts with the wider context. It could be intended as an agrofood zone that develops in and insists on a specific region. In this sense, it comprehends all the elements needed in feeding population (e.g., infrastructures and technologies, the inputs, biological processes, needed and outputs generated at each step research, and education and human resources). Therefore, it is strongly affected by economic, social, political, and environmental contexts (DuPuis & Goodman, 2005; Winter, 2003) and becomes then "a sociogeographic zone," which provides a more accurate definition as

actually site-specific, based on two main components: a socioeconomic relational and territorial component.

The Footprint of Food: The “Foodprint”

A city is not able to provide resources and feed entire population; this capacity comes actually from agricultural produce developed from the surrounding rural areas. According to ecogeographic system, the city tends to be surrounded by several rural areas within which livestock and agricultural activities providing the food. Nowadays, distribution is changed but this does not impact on dependence, at least in part, of the city. Urban growth determines a parallel increase in population needs and in demand for resources, or “metabolism” of a city. However, as this trend occurs, resources become more and more limited due to increased trade in transportation. The interaction among these factors is the basis for the determination of shape and spatial limit of a city, but it must be considered that its size determines, in turn, the amount of the demand for resources and then the area required.

Metropolitan AgriFood System

- Natural and cultural heritage sites that are unique and region specific
- Communities defined by cultural traditions, language, and dialect
- Production of goods and services which are mainly based on regional resources.
- “space of flows”
- Layer of electronic networks forming the basis for information and communication
- Layer of nodes and centers which locations follow primarily the functions of flow in the realm of decision-making (gated communities hotels, airports, company headquarters, etc.) as well as the principles of innovation in production and trade.
- Layer of the management and experts moving constantly between nodes and centers.

Ethics in Marketing and the Business Case for Sustainable Food Security

To accelerate their contribution to addressing sustainable food security:

- Greater sustainability and long-term food security—as opportunities for business success rather than solely as a way of reducing risks.
- Use the language of “resilience,” rather than solely “efficiency” and “cost reduction,” as a stepping stone to tackling bigger picture sustainable food security issues.
- Internalizing the urgency of the challenges. Quantifying risks and how they might evolve; by embedding sustainability into core strategy at all

levels of the company and by giving a senior manager in the company specific accountability for longer-term strategy.

- Developing a longer-term route map with milestones that are proportionate with the scale of the environmental and social challenges facing society.
- Building resilience in producer regions and developing closer long-term supplier relationships.
- Offering access to investment and training and setting up ethical intermediaries can improve producer resilience.
- Businesses that “unlock markets by providing market knowledge, risk capital and training to developing world producers, while helping retailers access new products and better manage their supply chains.”
- Working to ensure there is more effective pre-competitive collaboration. This might include challenging government—at UK and at EU levels.
- To ensure competition policy does not frustrate attempts by businesses to act collectively act on sustainability and long-term food security, particularly in relation to “commons” issues (for example fisheries).
- Demonstrating leadership and sharing best practice. This can mean: (1) pushing up minimum accepted standards within the industry (raising the ethical bar), (2) showcasing the viability of progressive companies’ sustainable business models
- Improving governance of resources. It applies particularly to common resources such as water and fish, which creates challenges and do not recognize boundaries, around usage rights and over the sustainable management of supply.

FAO’s Role in Urban Agriculture

Urban and peri-urban agriculture (UPA) can be defined as the growing of plants and the raising of animals within and around cities.

- UPA provides food products from different types of crops (root crops, grains, mushrooms, vegetables, and fruits), animals (rabbits, goats, poultry, cattle, pigs, guinea pigs, sheep, fish, etc.) as well as nonfood products (e.g., medicinal herbs, tree products, aromatic, and ornamental plants). It includes trees managed for producing fuel wood and fruits, as well as tree systems integrated and managed with crops (agroforestry) and aquaculture. It can make an important contribution to household food security, especially in times of crisis or shortages of food or natural calamities.
- Food products is either consumed by the producers, or sold in retail markets, such as the increasingly popular farmers markets found in many cities. Because locally produced food requires less refrigeration and transportation, it can supply nearby markets with fresher and more nutritious products at nominal prices. Consumers—especially limited income groups—enjoy easier access to freshly produce, better prices, and greater choice.

- FAO supports the transformation of UPA into a recognized urban land use and economic activity, integrated into National and local agricultural development strategies, food and nutrition programs, improve production, processing and marketing systems, and urban planning. It helps National and regional governments and city administrations optimize their policies and support services for UPA.

Social Impacts of Food Security

Environmental degradation, low agricultural productivity, high postharvest losses, limited connections to markets, energy poverty, limited education and nonagricultural opportunities, hunger, and thirst lead millions of desperate people to leave rural areas each year for the cities, only to find that life is often no better. Some of the Food entitlement failures are overcome by social protection responses which include (1) protect against harvest failure or livestock mortality by input subsidies, crop and livestock, (2) provide temporary employment by public work programs, (3) maintain market access to food and keep affordable for poor by food price stabilization, food subsidies, and grain reserves, and (4) reduce hunger/poverty by school feeding, supplementary feeding, conditional, and unconditional cash transfers.

The Need to Improve Food Safety and to Implement Quality Assurance

- The principles of food hygiene being the most successful means in protecting the consumer against food-borne health risks. The consumer's confidence in the safety of food is decreasing. Consumer from urban does not differentiate between commodities or diseases.
- Modern agriculture methods contributing to the increase of drug-resistant pathogens in humans. The latest and most serious attack is that of the Director General of the World Health Organization, who stated in his [Word Health Report \(1996\)](#): "...Making matters worse are modern types of food production.
- Antimicrobials are used in meat production to increase growth, but not sufficient concentrations to kill microbes. Drug-resistant microbes are then enters through the food chain to the consumer."
- Food safety issues increasingly used as marketing tools, nationally and internationally and can easily become nontariff trade barriers.
- The consumer has the right to ask for fresh and naturally raised (organic) products: The tendency "back to the farmers' markets" results in the increasing consumption of food that is not or less processed with several processing procedures (cleaning, packaging, canning, food additives such as preservatives, etc.) prior to marketing.

The “Preharvest” Food Safety and Quality Approach

Quality control is the assessment of a final product prior to marketing, i.e., it is based on quality checks at the production end aiming at assigning the final product to quality categories in order such as “low quality,” “regular quality,” “high quality,” and “nonmarketable.” As, at the end of the production chain there is no way to correct failures or upgrade the quality of the final product, low-quality products can only be sold at lower prices and the nonmarketable products have to be discarded. Production costs, however, as high as those of the high and regular quality products. Thus, quality control has only a limited capability to increase the quality and efficiency of a multistep production procedure.

Quality Assurance, in contrast to quality control, is the implementation of quality checks and procedures to immediately correct any failure which reduces the quality of the interim products at each and every production step.

Desired high quality final product is obtained by conducting:

Standard Operating Procedures which guarantee the desired quality of the products at every production step.

Good Manufacturing Practice: The management approach for long-term success through customer satisfaction, based on the participation of all members (suppliers included) in improving of the quality of the food.

Hazard Analysis Critical Control Point (HACCP) system is the internationally recognized system to assure safe food production. HACCP emphasizes prevention in the avoidance of problems in food safety. HACCP combines common sense with an evaluation of risks to identify the points along the food production chain, where possible hazards may take place, and then to monitor and strictly manage those points to make sure the process is under control.

The HACCP system is critically made into the following three parts:

1. Identification of hazards, and the determination of the severity of the hazard and risks (growing, harvesting, processing, distributing, and preparing) and/or using a raw material or food product. Hazard usually means the contamination, growth or survival of microorganisms related to food safety or spoilage. A hazard can also include foreign objects (glass or metal fragments) or dangerous chemical contaminants.
2. The determination of critical control points (CCP). A CCP is a location, practice, procedure, or process which can be used to prevent or minimize unacceptable contamination, survival or growth of spoilage organisms or food-borne pathogens or introduction of unwanted chemicals or foreign objects.
3. Establishment and implementation of monitoring procedures to determine that CCP is under control. Monitoring systems must be able to effectively

recognize if a CCP is under control. Corrective measures must be defined to be used when a CCP monitoring point shows that the system is out of control.

Improving Nutrition Outcome Requires More Than Food

The definition of food security includes nutrition language as an essential part of food security and itself is only one aspect of achieving optimal nutrition. Food security is necessary, but not sufficient for nutrition security (Jones, Ngure, Pelto, & Young, 2013). Modified food security and nutritional security requires multiple disciplines and sectors (sectors including agriculture and food, health, education, environment, water and sanitation, and women's empowerment) to demonstrate its impact on food and nutrition security.

The Most Vulnerability From an Under Nutrition Perspective

In the field of nutrition, it is important to understand who is most vulnerable to malnutrition in terms of under nutrition and overweight. Moreover, it is very important to understand the drivers of susceptibility and the consequences causes. The nutritional needs of children under 2 years of age are critical for growth (Adair et al., 2013). Typically, malnutrition occurs due to increased nutrition requirement at specific point of life cycle, particularly during young children stage, adolescent girls, pregnant, and lactating women (Fig. 3.1). The drastic or malnutrition or growth flatterer occurs at the age of 6–24 months; child is not protected by exclusive breastfeeding and leads to the state for exposure for infectious disease through contaminated food and water. Even some evidences suggest that, nourishing after 24 months of age also unlikely to recover growth lost in the first 2 years from the malnutrition's (Victora, de Onis, Curi Hallal, Blössner, & Shrimpton, 2010).

The Intractable, Equity Debate on Sustainable Diets

Types of food what we eat are changing day by day and driving a new demand for new types of food grown and processed in particular ways. As population increases in particularly India, China, and Brazil, diets not only shift toward higher quality, nutrient-dense products such as meat, dairy products, and oils but also toward more ultraprocessed foods. Globally, we are recognizing that the health of human beings cannot be isolated from the health of ecosystems (Johnston, Fanzo, & Cogill, 2014). The Global Food Security Index consider the core issues of availability, affordability, quality, and safety across a set of 109 countries based on environmental conditions supporting to the agriculture and way of people adopting the agriculture in daily life. Lot of water needed to produce nutritious food products for

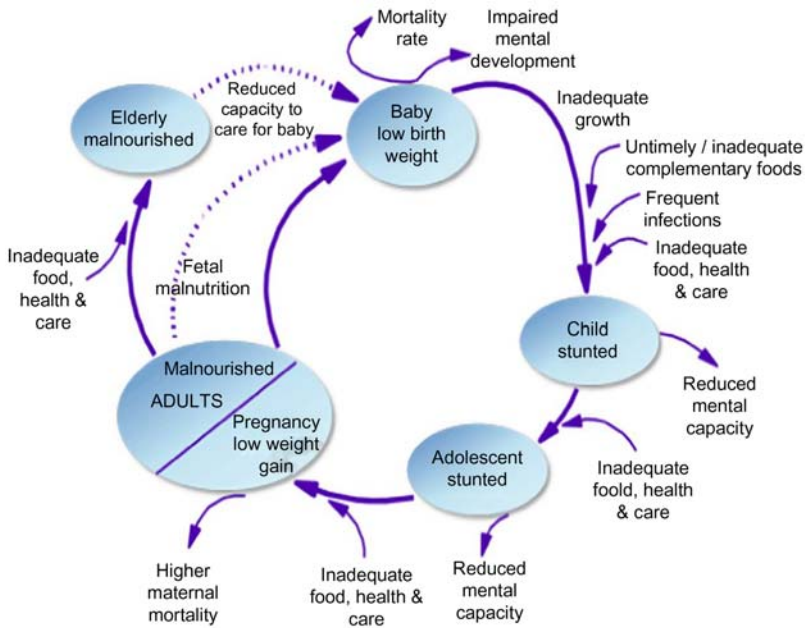


FIGURE 3.1 Different phases of poor nutrition and its effects on human life.

consumption, is one of the important matter to discuss and to be maintained at high priority than what we eat. At the heart of sustainable diets are animal-source foods. Animal source can provide different micronutrients that are very difficult to get from plant sources especially calcium, zinc, iron, vitamin A, Vitamin-B12, and riboflavin (Dewey & Adu-Afarwuah, 2008). That's why most of the countries shifting diets from plant based to refined foods include meat and dairy milk except the poor countries that cannot afford the leap (Popkin, Adair, & Ng, 2012; Wilkinson et al., 2009).

Climate Change Adaption and Mitigation

Climate change is challenging to the farmers on every continent to deal with altered weather patterns, novel agricultural pests, and new water conditions. These challenges will be felt most intensely by smallholders in some of the poorest regions of the world. Climate change calls for new approaches to sustainable development that take into account complex interactions between climate, social, and ecological systems (Fig. 3.2). Climate-resilient pathways are trajectories that combine adaptation and mitigation to realize the goal of sustainable development. It stresses that equitable economic development is key to addressing environmental problems both in developing and developed

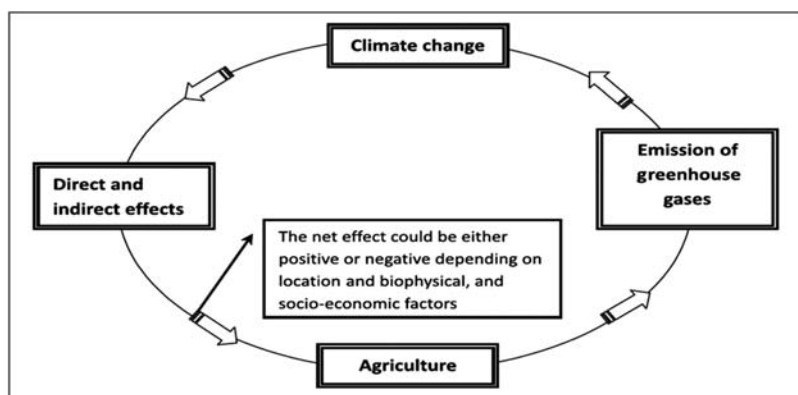


FIGURE 3.2 Interrelationship between climate change and agriculture.

countries in ways that are sustainable for the long term (Halsnaes, Shukla, & Garg, 2008; Lafferty & Meadowcroft, 2010).

Mitigation is recognized to be important for sustainable development in two ways. First, it reduces the rate and magnitude of climate change, reduces climate-related stresses on sustainable development, including effects of extreme weather and climate events (Lenton, 2011). The challenge for climate-resilient pathways is to identify and implement mixes of technological and governance options that reduce net carbon emissions and at the same time support sustainable economic and social growth in a context where rising demands for economic and social development need to be combined with technology transitions without disrupting the development process. Climate change responses include adaptation mitigation and integrated mitigation. Generally considered a separate response issue is “geoengineering.” Geoengineering states that, if climate change mitigation is not sufficiently successful, policymakers may be faced with demands to find further ways to reduce climate change and its effects.

FUTURE OF FOOD AND FARMING: CHALLENGES AND CHOICES FOR GLOBAL SUSTAINABILITY

The impact of modern agriculture on natural resources has become a major global concern. Population growth and expanding demand for agricultural products constantly increase the pressure on land and water resources. A major point of concern for many intensively managed agricultural systems with high external inputs is the low resource-use efficiency, especially for nitrogen. A high input combined with a low efficiency ultimately results in environmental problems such as soil degradation, eutrophication, pollution of groundwater, and emission of ammonia and GHGs. Evidently, there is a need for a transition of current agricultural systems into highly resource-use

efficient systems that are profitable, but at the same time ecologically safe and socially acceptable. Gradual rising in the temperatures globally and decrease in fresh-water availability posing critical challenges for agricultural researcher in order to increase crop performance under suboptimal conditions. Recent advances in our knowledge about plant responses to stress conditions and improving molecular tools for plant breeding results in the introduction of new drought-resistant varieties, both GM (Genetically modified) and nonGM crops. In present scenario of developed agricultural practices, inefficient use of fertilizers and other chemicals are the major sources of pollutants in air, land, and water, which are highly toxic to people and wildlife. Greatest challenge of the 21st century, transitioning to more sustainable agricultural practices while increasing the food and feed supply, at the same time to minimize the negative effects on agricultural productivity like warming climate. Food availability refers to the physical existence of food. On national level, food availability is a combination of domestic food production, commercial food imports and exports, food, and domestic food stocks. On household level, food could be from own production or bought from the local markets.

Future challenges to be addressed in the following areas for sustained agriculture and food security:

Important drivers affecting the food system are, increase in the global population, changes in the size and nature of per capita demand, climate change, enhancing photosynthesis, reducing environmental impact, defeating exotic diseases, exploiting genome advances, understanding diet and health, competition for key resources and changes in values and ethical stances of consumers, and also methods in the governance of food system at both national and international levels. Balancing the sustainable future demand and supply of foods, improving productivity using existing/new tools and techniques of Science and Technology and address new threats, reducing waste. Addressing the threat of future volatility in the food system, ending hunger and meeting the challenges in reducing the GHGs are also important. Implementation of strategic policies in maintaining ecosystem and biodiversity for sustainable food production is necessary. It is also necessary to promote sustainable agriculture to safeguard the economic viability of the farmers. Sustainable agriculture is a set of farming practices which can continue to maintain the farm productivity, efficiency and profitability in the long run, without depleting the natural resources and the environment. For ensuring the sustainability of small farmers, it may be useful to encourage the adoption of indigenous skills, use of internal inputs, preferably from organic sources, least dependence on external inputs, greater emphasis on crop diversity, symbiotic crop rotation, and production focused on local needs and easy marketability.

CONCLUSION

With the global population expected to top 9 billion people by 2050, and given the demands for more protein-rich diets by populations with increasing incomes, farmers around the world will be hard pressed to meet demand. Between 1970 and 1990, global aggregate farm yield rose by an average of 2% each year, largely due to the Green Revolution and focused investments in research and technology. Food security exists when all people, at all times, have physical and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life. Since 1990, aggregate farm yield growth has stagnated and even reversed course in some areas. Increase in food production does not automatically ensure food security, if the poor do not have the power to buy. Replacement of local seeds with change of crops, certified seeds, and use of mineral nutrients, improved farm implements, installation of irrigation system, and plant protection measures are some of the new initiatives to enhance the crop yields. Climate change is a repeated force of livelihood change, as increases in unpredictable and unusual weather patterns affect communities globally.

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Chapter 4

Plant Biotechnology and Crop Improvement

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INTRODUCTION

Food security, human health improvement, and environmental sustainability are the prime challenging issues of our time. The latest estimate of UN-DESA shows that the world population is going to cross 9.6 billion by 2050, a surprising increase of 0.5 billion over the previous estimate of 9.1 billion (Jaggard, Qi, & Ober, 2010). Presently, over 2.3 billion people live with less than 2500 kcal, while the other 1.9 billion consumes about 3000 kcal (Alexandratos & Bruinsma, 2012). The future agriculture, therefore, needs to feed not only the hungry 9.6 billion, but has to provide quality

food to sustain a healthy nourished world population despite having decreasing land, water, and other primary input resources. Adverse effects from climate change are expected to escalate food price further, increasing the unavailability of food to poor people. With a simultaneous increase in per capita income, demand for agricultural products is sure to increase, requiring crop productivity to be increased by at least 1.5–2.0 times for the major crops (Rosegrant et al., 2014). In addition, CO₂ concentration is expected to increase by 32% with global temperature increase of 1.8°C by 2050 (Jaggard et al., 2010). Although the increased CO₂ concentration is expected to increase productivity of C3 crops by 13%, this gain would be negated by warmer climate.

Sustainable development is considered as the best practice for the survivability of future generations while ensuring food and nutritional safety (Webb, 2014). The issue of sustainability has surfaced time and again when natural systems are overexploited. In fact, the term “sustainability” was first used in relation to deforestation in Germany (du Pisani, 2006). Intensive agriculture has overexploited natural resources to meet our growing demands. While controlling human population which is a serious issue and the root cause of most of the problems, serving sufficient and quality food to all the hungry mouth is a basic necessity that has driven agriculture to become more intensive, productive, and exploitative.

To find plausible solutions for improving crop productivity without further destabilizing the environment is a daunting task, more so when some of these problems are results of overexploitation of scientific developments. Plant biotechnology or green biotechnology, a leading frontier of plant science, provides cutting-edge tools and technologies for finding reliable solutions to many of these problems, empowering policy makers to devise achievable strategies for sustainable development. As a global initiative, the United Nations fixed eight Millennium Development Goals (MDGs), where the number one goal was to eradicate poverty and hunger (<http://www.un.org/millenniumgoals>). Initial target of reducing hungry population by half has been achieved by 2015, a major credit of which goes to new age breeding programs powered with biotechnological tools for higher selection efficiency that allowed continuous increase in global crop production, under both favorable and adverse agro-ecosystems (Park, McFarlane, Phipps, & Ceddia, 2011), putting a confidence on these technologies for sustainable development. While designing a charted path for sustainable development, the United Nations have further set 17 sustainable development goals (SDGs) (<https://sustainabledevelopment.un.org>) to develop a better living world. The first two goals set ambitious targets to end poverty, hunger, and malnutrition by 2030, to double productivity of small scale farmers as well as to ensure a sustainable production system. To meet these challenges, innovative approaches are required to be integrated with conventional crop improvement strategies (Fig. 4.1). Fortunately, scientific discoveries are not

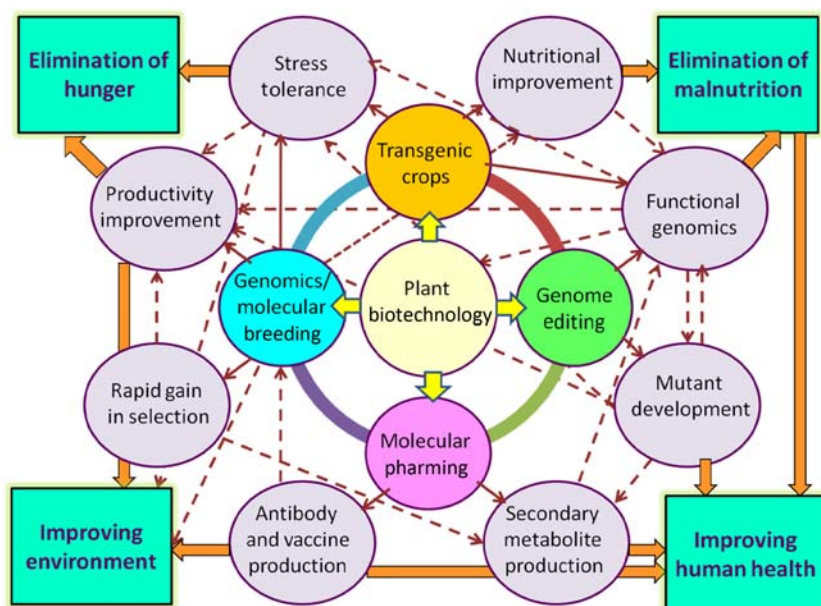


FIGURE 4.1 Applications of plant biotechnology in different frontiers of crop improvement for fulfilling millennium and sustainable development goals.

stagnant, and new efficient technologies and products are coming up to provide help for devising realistic strategies to tackle the challenges of food production and nutritional security. Our discussion in the following sections will introspect into biotechnological applications in crop improvement including genetic engineering, molecular breeding, molecular pharming, and genome manipulation technologies with the emphasis on major research achievements and commercial applications that hold great promise to design paths of success toward accomplishing targets of sustainable development.

BOOSTING SUSTAINABLE CROP PRODUCTION THROUGH BIOTECHNOLOGICAL APPROACHES

Targeting yield improvement of crop plants is bidirectional: one aiming to increase the inherent potential of crop plants to produce more under optimal crop management practices, while the other aims to enhance the sustainability of the production under stress conditions. A combination of these two is highly desirable in developing the ideal genotype having higher yield, stability, and adaptability over diverse climatic conditions. A third approach, which is not linked with direct change in crop genotype, is to increase the quality of the environment, so that the plants will thrive better and produce more. Biotechnology contributes to enhance crop productivity in all of these

approaches, by assisting in the selection of superior genotypes in breeding program, by modifying crop genotype or by improving the environment. With new tools and techniques, genotype assessment and genotypic selection have become more precise and efficient, allowing screening of thousands of plants for desirable allele within a short time frame as well as enabling researchers to increase breeding value of a trait through efficient selection. Inarguably, these approaches have aided in sustainable agricultural practice by minimizing requirement of one or more input resources, though there is a general perception that transgenic technologies are not part of sustainable agriculture. In this section, we will concentrate mainly on development of transgenic crops for imparting resistance to biotic stress, tolerance to herbicides, and modification of nutritional quality, with a quick glance on established and novel molecular breeding technologies that are going to dominate the area of crop biotechnology in upcoming decades.

Enhancing Productivity Through Genetically Modified Crops

Protecting Plants From Diseases

A major emphasis of transgenic crop development is directed to developing host resistance to diseases. During 2001–03, plant pathogens caused 12.5% loss in production of six major crop species (wheat, rice, maize, potato, soybean, and cotton) worldwide, in spite of the fact that the use of chemical control measures has reduced potential yield loss by 32% (Oerke, 2006). Chemical control of diseases has inherent environmental problems, thus the development of host resistance is a priority for sustainable environment friendly crop protection.

Resistance to Viral Pathogens

Viral pathogens cause substantial crop loss in beans, potato, tomato, papaya, alfalfa, vegetables, and fruit crops. Various strategies for resistance to viral diseases have been employed including encapsulation of viral genome, silencing of viral genes by antisense RNA or by small RNA molecules, expression of ribozyme, or modification of host factors. Expression of viral coat protein (CP) coding genes in host plant allows encapsulation of the invading viral DNA by the CPs within host cell, thereby preventing expression of the viral genes in host. The strategy was first developed by engineering CP gene of tobacco mosaic virus (TMV) into tobacco through which the transgenic plants delayed TMV symptom development (Powell-Abel et al., 1986). Soon it was used to develop resistance to alfalfa mosaic virus, an insect transmitted virus of alfalfa (Loesch-Fries et al., 1987). CPMR is widely utilized to develop transgenic crops resistant to several viral diseases, such as against papaya ringspot virus (PRSV), cucumber mosaic virus (CMV), potato virus X and Y (PVX, PVY), rice tungro virus (RTV), rice

ragged stunt virus (RGSV), wheat streak mosaic virus (WSMV), sugarcane yellow leaf virus, barley yellow dwarf virus (BSWV), bean pod mottle virus (BPMV), beet yellow virus, alfalfa mosaic virus, and many other viruses (Hoekema, Huisman, Molendijk, van den Elzen, & Cornelissen, 1989; Vinogradova, Kamionskaya, Zinovkin, Agranovsky, & Skryabin, 2012). CPMR has been commercially very successful; in 1995 Asgrow company obtained permission of commercial cultivation of squash transgenic “Freedom II,” having resistance to zucchini yellow mosaic virus and watermelon mosaic virus II. Transgenic papaya cultivars resistant to ringspot virus saved the papaya industry of Hawaii, which was severely affected during 1990s (Gonsalves, 2006).

In addition to CP genes, other viral genes such as replication related proteins, movement proteins, nuclear inclusion protein, and nonspecific RNA sequence have been introduced into host genome for obtaining resistance to viral pathogens. The mechanism of such resistance was later elucidated due to posttranscriptional gene silencing (PTGS) mediated by viral RNA expressed in the host. The viral RNA expressed in the host genome is converted into a dsRNA, which then forms a gene silencing complex by the interaction with proteins and targets the viral genome for modification (Duan, Wang, & Gao, 2012). Viral sequences like small hairpin RNA having inverse repeats increase the efficiency of VIGS in transgenic plant (Wesley, Helliwell, Smith, et al., 2001). While VIGS is a valuable tool in functional genomic analysis, it has also been successfully exploited to develop resistance against MYMV, RTV, potato spindle tuber viroid, bean golden mosaic virus, and banana bunchy top virus (Aragao & Faria, 2009; Pooggin, Shivaprasad, Veluthambi, & Hohn, 2003; Shekhawat, Ganapathi, & Hadapad, 2012). VIGS has also been used for resistance against fungal diseases as well as insect pests, including resistance to powdery mildew in barley (Várallyay, Giczey, & Burgyán, 2012), wilt in pepper (Anu, Jessymol, Chidambareswaren, Gayathri, & Manjula, 2015), cotton bollworm (Mao, Tao, Xue, Wang, & Chen, 2011), and abiotic stress tolerance (Bao et al., 2015). Another similar strategy is to express miRNA sequences designed to interfere with the target gene. Using artificial miRNA constructs, resistance has been developed against turnip yellow mosaic virus in *Arabidopsis*, against potato virus X and potato virus Y in tobacco, and against leaf curl virus in tomato.

Resistance to Bacterial Pathogens

Bacteria cause a number of serious diseases in crop plants; notable examples are bacterial blight of rice, bacterial wilt of vegetables, fire blight of apple, and canker disease of citrus. The major strategies for resistance to bacterial pathogens in crop plants include deployment of resistance (R) genes, production of antimicrobial peptides, introduction of genes for bacterial toxin

tolerance, inhibition of bacterial pathogenesis, and biosynthesis of other defence-related proteins.

Genetic engineering of R-genes for resistance to fungal disease is primarily limited within species or transferred from related species. Several R genes like *Rpg1* of barley, *Rps1-k* from soybean, *Rpi-blb1* and *Rpi-blb2* of *Solanum bulbocastanum*, *Rps1-k* of soybean, and *Hm1* of maize have been engineered in the same species. R-genes effective against late blight in potato were first identified in wild relative *S. bulbocastanum*, and transferred to a number of commercial potato varieties, including popular cultivar Russet Burbank (Kuhl, Zarka, Coombs, Kirk, & Douches, 2007). However, the *Rpi-blb1* gene could only confer resistance to foliar blight but was not very effective for preventing tuber infection. Commercial exploitation of these genes has led to the development of transgenic potato varieties like “Fortuna” by BASF, but due to regulatory delays the product has been withdrawn. Soybean *Rps1-k*, which provides resistance to *Phytophthora sojae*, has also been transformed into susceptible varieties for enhanced resistance (Gao, Narayanan, Ellison, & Bhattacharyya, 2005).

In rice, resistance to bacterial blight has been obtained by transgenic expression of endogenous *Xa21* gene and other *Xa* genes. Under the International Program on Rice Biotechnology, *Xa21* has been integrated into elite *indica* and *japonica* rice varieties and the progeny lines were used to develop resistant varieties in different countries (Tu et al., 1998). Transgenic rice containing the *Bar* gene for resistance against herbicide Biolophos also provides resistance to sheath blight disease. Majority of the R-genes are species specific and are generally transferred through phenotype-based or marker-assisted backcross breeding (MABB) rather than by genetic engineering. *Xa21*, however, has been successfully transferred across species to provide resistance against other *Xanthomonas* species, such as against *Xanthomonas axonopodis* pv. *citri* in citrus and *Xanthomonas campestris* pv. *musacearum* in banana (Mendes et al., 2010; Tripathi, Lorenzen, Bahar, Ronald, & Tripathi, 2014).

Several antimicrobial peptides like attacin, magainin, defensin, and cecropin have been transferred in many fruits, vegetables, and forest tree species including potato, tomato, tobacco, grape, orange, apple, elm, and poplar for resistance to bacterial and fungal pathogens. Expression of attacin, cercopin, and their analogs in apple provided resistance to fire blight (Liu et al., 2001), whereas in sweet orange attacin effectively prohibited citrus canker (Boscardioli et al., 2006). A transgenic tomato line containing synthetic magainin gene exhibited considerable resistance to *Pseudomonas syringae* (Alan, Blowers, & Earle, 2004). Lysozyme, another antimicrobial peptide, degrades the murein layer of peptidoglycan in bacterial cell wall, thus causing lysis. Transgenic tobacco, potato, and apple containing lysozyme gene show resistance to several bacterial pathogens. Many bacterial pathogens also produce exopolysaccharides (EPS), which contribute to in pathogenicity and

protection of bacteria. Bacteriophages, on the other hand, produce EPS-depolymerase to degrade the EPS during phage infection. Engineering EPS-depolymerase from bacteriophage to apple resulted in resistance to fire blight in apple (Borejsza-Wysocka et al., 2007).

Resistance to Fungal Pathogens

Fungal diseases are most serious groups of diseases of crop plants, causing loss of billions of dollars worldwide. Diseases like late blight of potato, head blight of cereals, sheath blight and blast of rice, leaf blight in maize, wilt and rots in vegetables, and powdery mildew of grapes are some of the major fungal diseases. Strategies for resistance include degradation of the fungal cell wall, production of antifungal toxins, exploitation of resistance genes, overexpression of genes that are induced during pathogen response, breakdown of fungal metabolites involved in infection and inhibition of growth of the fungi by limiting essential nutrients.

A potent strategy for fungal disease resistance is to synthesize genes that can degrade fungal cell wall. Chitinase and β -1,3-glucanase are cell wall degrading enzymes that specifically disrupt fungal mycelium by degrading chitin and glucan, respectively. Chitinase from barley, tobacco, petunia, bean, and rice has been exploited for resistance (Punja, 2001; Tabei et al., 1998). Rice chitinase, in particular, has been transferred to many crops including wheat, peanut, tomato, grape, eggplant, banana, and cucumber with significant resistance against fungal pathogens. Since chitinase is already present in crop plants, overexpression or modification of endogenous chitinase is another strategy for inducing resistance. Overexpression of chitinase and oxalate oxidase has resulted in enhanced resistance against sheath blight in rice (Baisakh et al., 2001; Karmakar et al., 2016). In addition to plant sources, chitinase from fungal species, particularly from *Trichoderma*, is also used for transgenic disease resistance (Cheng et al., 2015).

Various other PR proteins and phytoalexins are induced during disease development; introduction or overexpression of these genes can induce disease resistance. Defensins are small cysteine-rich antimicrobial PR-proteins being ubiquitously present in microbes, plants, and animals. These small peptides show high antimicrobial activity, particularly against fungal pathogens. Transgenic cotton carrying defensin *NaD1*, from *Nicotiana glauca*, showed higher tolerance to *Verticillium* wilt and *Fusarium oxysporum* f. sp. *vasinfectum* along with twofold increase in yield (Gaspar et al., 2014), suggesting high commercial potential for this transgenic. Seo et al. (2014) engineered a defensin in pepper for developing resistance against *Colletotrichum gloeosporioides*. It was observed that in the transgenic lines jasmonic acid-biosynthetic genes and pathogenesis-related genes were overexpressed, leading to resistance against fungal pathogenesis. A wheat defensin was found to accumulate under cold acclimation, which provides

resistance against snow mold fungus, *Typhula ishikariensis*, a major pathogen of winter wheat (Sasaki et al., 2016). Transgenic wheat containing defensin gene also shows resistance to *Fusarium* head blight.

Breakdown of fungal pathogenicity associated metabolites is another effective approach for delaying disease development. Many fungi produce oxalic acid, which acts as a pathogenicity factor. Breakdown of oxalic acid by expressing enzymes like oxalate oxidase, oxalate decarboxylase, or oxalyl-CoA-decarboxylase induced resistance against *Sclerotinia* in soybean, tomato, tobacco, sunflower, and lettuce as well as against *Septoria* in tree species. Another similar strategy is to inhibit growth of the fungus by limiting the supply of elements required for growth. For example, the rice blast fungus requires biotin for its growth. By engineering a gene *tamavidin1*, which binds to biotin and limits its availability, resistance to blast disease has been developed in rice (Takakura, Oka, Suzuki, Tsukamoto, & Ishida, 2012).

Pyramiding Transgenes for Disease Resistance

Gene pyramiding for multiple disease resistance is an efficient strategy for broad spectrum durable resistance in crop plants. Chitinase has been used with several other genes for pyramiding broad spectrum resistance. Two approaches are more popular, introducing multiple genes against one pathogen for resistance against different strains (pyramiding against a disease) or stacking of different genes for combined resistance against different pathogens, pests, or combinations thereof. Transgenic pyramiding of thaumatin-like protein and *Xa21* in rice have resulted in higher resistance to both bacterial blight and sheath blight (Maruthasalam, Kalpana, Kumar, et al., 2007). Combined expression of chitinase from *Streptomyces olivaceoviridis* and glucanase from barley in pea resulted in higher resistance to fungal spore germination (Amian, Papenbrock, Jacobsen, & Hassan, 2011). Similarly, pyramiding of fungal chitinase and plant cystatin genes in tomato provided resistance against nematode diseases and coexpression of chimeric chitinase from *Trichoderma atroviride* and polygalacturonase-inhibiting protein from *Phaseolus vulgaris* in transgenic canola enhanced resistance up to 44% against *Sclerotinia sclerotiorum* (Ziaei, Motallebi, Zamani, & Panjeh, 2016). Combination of chitinase and defensin genes also provided better resistance to *S. sclerotiorum* in *Brassica napus* (Zarinpanjeh, Motallebi, Zamani, & Ziaei, 2016).

Stacking of chitinase, glucanase, and ribosome inactivating protein (RIP) genes in rice has resulted in resistance against blast and sheath blight, two most serious diseases. Pyramiding of defensin and chitinase genes in transgenic potato also provided resistance against a number of fungal pathogens (Khan et al., 2014). In another study, genetically transformed tobacco carrying chitinase and protease inhibitor genes in plastid genome exhibited broad spectrum resistance against diseases, insects, and abiotic stresses (Chen et al., 2014).

Insect Resistance

Insects are the second most serious threat to crop cultivation after weeds, causing about 18% crop loss worldwide. Over a period of 1964–2003, production loss due to insect pests has remained almost constant in maize (9.6%–12.5%), wheat (5.0%–9.3%), and cotton (11%–12.3%), indicating that despite several control measures, the effective crop loss could not be reduced (Cramer, 1967; Oerke, 2006). Insect protection is almost entirely dependent upon chemical control, without which potential crop loss would have risen to 24% of the total production. Development of host resistance by transgene integration is one of the most successful examples of sustainable transgenic strategies for reducing crop loss as well as pesticide use; use of insect resistant transgenic crops has reduced the use of chemical pesticide by 37% (James, 2015).

Bt-Toxin Engineering

Bacillus thuringiensis (Bt), a Gram-positive, rod-shaped sporulating soil bacterium, is a well-known insect pathogen. The bacterium produces a wide variety of insecticidal toxin proteins, which makes it an efficient biological control agent. It synthesizes an insoluble crystalline (cry) toxin proteins, known as δ -endotoxin, which bind to receptor on the epithelial membrane of insect midgut, causing disruption of osmotic balance and insect cell lysis. Bt-toxin is more specific for lepidopteran and coleopteran pests, but some cry genes like *cry2*, *cry3A*, *cry4*, *cry4Aa*, and *cryIIAa* are active against hemipteran pests. Modified and fused cry toxins also show increased activity. However, the control of hemipteran pests using these toxins did not bring much success.

With the first engineering of *cry* gene in tobacco in 1987, more than 800 endogenous and synthetic *cry* genes have been reported (Crickmore et al. 2016). Potato was the first commercialized transgenic Bt-crop; a transgenic potato expressing *cry3A* protein was branded as “NewLeaf” that provided resistance to Colorado potato beetle. Bollgard, the famous Bt-cotton, developed by Monsanto was approved for cultivation in 1996 (Jones et al., 1996) along with herbicide resistant cotton, which rapidly increased area under GM cotton in the United States followed by other countries. Today GM cotton is cultivated worldwide occupying 78% of the total global cotton area being commercially cultivated in the United States, China, India, Argentina, Brazil, Mexico, Colombia, Australia, South Africa, Pakistan, Burkina Faso, Myanmar, Sudan, and Costa Rica (James, 2015). The first field trial of transgenic Bt-rice was performed in 2001; two rice varieties, Minghui 63 and Shanyou 63, showed high insect resistance with no yield compensation (Tu et al., 2000). Bt-rice received biosafety certificate in 2009 in China, but the approval for commercial cultivation is still pending.

As a second generation Bt-technology, gene pyramiding has been adopted as a major strategy to broaden the effectiveness and range of resistance. The commercial release of Bollgard II from Monsanto marks the initiation of cultivation of second generation *cry* gene pyramided Bt-crops (Perlak et al., 2001). *Cry* genes have also been integrated with other insect resistance genes and other traits like herbicide tolerance (HT) and disease resistance. In rice, Zhao et al. (2004) pyramided Cry1Ac and cowpea trypsin inhibitor for enhanced resistance to *Chilo suppressalis*, while Jiang et al. (2004) combined *Xa21* with *cry1Ab/Ac* for broad spectrum resistance to insects and bacterial blight. In chickpea, pyramided *cry1Ab* + *cry1Ac* and fused *cry1Ab/Ac* gene provided better resistance to *Helicoverpa armigera* (Ganguly, Molla, Karmakar, Datta, & Datta, 2014; Mehrotra, Singh, Sanyal, Altosaar, & Amla, 2011). Presently stacked traits, particularly combination of HT and insect resistance, occupy 28% of the global transgenic area (James, 2015).

Vegetative Insecticidal Proteins

B. thuringiensis also synthesizes another group of insecticidal proteins during the vegetative growth phase known as vegetative insecticidal proteins (Vip) (Estruch et al., 1996). Similar to Cry proteins, Vip kills insects by binding to the midgut, but the mechanism of action is different. Commercial transgenic maize stacked with Bt and Vip are marketed by Syngenta under the brand name Viptera, Agrisure, and Duracade. A synthetic vip gene (Syn vip3BR) was engineered in chloroplast genome of rice mega-cultivar Swarna by Pradhan et al. (2016) using a Cre/lox recombination system to develop marker-free transplastomic GM Swarna, which exhibited high resistance to stem borer, horn caterpillar, and leaf folder.

Plant Lectins

Many plant lectins exhibit toxicity to insect pests by binding with the sugar moieties present in the insect midgut, causing agglutination. Lectins bind to carbohydrates to form glycoconjugates. Snowdrop (*Galanthus nivalis*) lectin (agglutinin), PTA (*Pinellia ternate*) lectin (agglutinin), herein-related lectins, jackalins, ricin-related lectins, and lectins from legumes are toxic to insects. The snowdrop lectin received early attention as it is toxic against sucking pests, where Bt-toxins are not working (Hilder et al., 1995; Shi et al., 1994). It has been engineered in rice, tobacco, potato, wheat, and maize for resistance to brown planthopper, green leafhopper, whiteback planthopper, and aphids (Wang, Zhang, Sun, Tang, & Zhang, 2005; Yu, Wang, Huang, Ma, & Xia, 2014). PTA lectin has been engineered in tobacco and wheat for aphid resistance (Jin, Zhang, & Daniell, 2012; Yu & Wei, 2008). Pyramiding of different lectin genes has also been successful. Pyramiding of snowdrop lectin and *Allium sativum* lectin provided higher resistance to BPH, GLH, and

WBPH than single gene transgenic rice lines. In addition to insect resistance, lectins also provide resistance to fungal as well as bacterial pathogens. Lectins are also used as carriers for other toxins for better penetration. For example, spider venom toxin is fused with snowdrop lectin, which becomes more active in the insect gut. Transgenic *Arabidopsis* with such fused construct exhibited higher resistance to *Myzus persicae*, a cosmopolitan aphid species, and also to *Sitobion avenae*, wheat aphid.

Other Strategies for Insect Resistance

Protease inhibitors (PIs) are widely distributed in plants providing protection against insect pests and pathogens. These inhibitors bind to the digestive proteases in insect and inhibit proteolytic activities. These compounds are more effective against feeding insects than sucking insects. Trypsin inhibitors have been the most targeted group of PI for transgenic insect resistance development, of which cowpea trypsin inhibitor holds good promise. As early as in 1987, cowpea trypsin inhibitor was engineered in tobacco for resistance to *Heliothis virescens* (Hilder, Gatehouse, Sheerman, Barker, & Boulter, 1987). However, insect pests tend to develop resistance to PI, which limits the use of PI-transgenic crop development. Pyramiding of PI genes has been more successful for overcoming insect resistance. Overexpression of potato type II inhibitor (PIN II) and carboxypeptidase inhibitors (PCIs) in tomato provided resistance to *Heliothis obsoleta* and *Liriomyza trifolii*. A combination of potato type I inhibitor and *N. alata* proteinase inhibitor (NaPI) in transgenic cotton provided resistance against *H. armigera* with increased boll yield (Dunse et al., 2010). Combination of Cry1Ac and cowpea trypsin inhibitor was also promising. Similarly, transgenic potato leaves expressing both oryzacystatin I and II were less desired by Colorado potato beetles than non-transgenic control. However, long-term feeding revealed similar proteolytic activity in insects fed with leaves of transgenic and control plants (Cingel et al., 2016). New PIs such as clitocypin from mushroom or subtilisin-like proteases are being tested for efficacy against insect pests, which may hold promise for insect control through PI.

Insects also produce chitinase for shedding exoskeletons. The first report of using insect chitinase came in 1996, when chitinase from *Manduca sexta* was engineered in tobacco to provide resistance to merchant grain beetle *Oryzaephilus mercator*. The *M. sexta* chitinase has also been engineered in papaya for resistance to spider mite. Also, chitinase from *Spodoptera littoralis* has been engineered in maize to induce resistance against corn borer (*Sesamia cretica*). Antimicrobial peptides like defensins also increase resistance against insects. Choi et al. (2009) transferred a defensin gene from *Brassica rapa* to rice for inducing resistance to brown planthopper. Inhibition of insect neuropeptides that regulate insect feeding behavior is another novel approach for controlling insect pests. A neuropeptide F (NPF)

was identified from cotton budworm *H. armigera*, and a dsNPF was transferred in tobacco and cotton to block the expression of NPF of *H. armigera*, resulting in lower body weight of insects (Yue et al., 2016).

Herbicide Tolerance

One of the most commercially successful applications of transgenic plant development is to develop HT in crops. In 1994, HT tobacco was approved for cultivation, while in 1996 HT in soybean, cotton, and maize was approved. Today, 94% of the soybean, 89% of the cotton, and 89% of the maize grown in the United States are genetically modified for HT (USDA-ERS, 2016). This has remarkably increased the use of glyphosate with a 15-fold increase in use since 1996, which is of significant concern for the environment (Benbrook, 2016).

Glyphosate Tolerance

Of the various transgenic herbicide resistant crops, glyphosate resistant crops have been the most rapidly adopted transgenic technology, being commercially used in soybean, maize, canola, cotton, and other crops. These crops were modified to be resistant to the broad-spectrum herbicide glyphosate (Roundup) thus the prefix “Roundup-Ready” is used for these HT transgenic crops. Glyphosate is a nonselective herbicide which inhibits the synthesis of aromatic amino acids from shikimate-3-phosphate by inactivating the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase through competitive inhibition. The herbicide competes with one of the substrates, phosphoenol pyruvate (PEP), and stops the enzyme activity by stable EPSP–glyphosate complex. This also affects downstream pathways including phenylpropanoid and flavonoid pathways, killing the plant effectively. Transgenic crops resistant to glyphosate carry a glyphosate insensitive EPSP synthase, coded by *aroA* gene from *Salmonella typhimurium*, maintaining normal activity in the transgenic plant (Comai, Sen, & Stalker, 1983). Search for different *aroA* genes from various organisms resulted in two classes of *aroA* enzymes, class II providing higher tolerance than class I enzymes. Comparative evaluation of *aroA* genes (*G2 aroA*, *HTG7 aroA*, *A1501 aroA*, *RD aroA*, and *AM79 aroA*) from different bacterial sources showed that transgenic plants expressing class I *AM79 aroA* were more tolerant to glyphosate (Cao et al., 2012). Recently, the introduction of a novel *aroA* gene from *Janibacter* sp. provided fourfold tolerance to Glyphosate in rice (Yi et al., 2016). As an alternate approach, glyphosate oxidoreductase (*gox*) gene from *Ochrobactrum anthropi* has also been used to break down glyphosate into aminomethyl phosphonic acid and glyoxylic acid (Reddy, Duke, & Rimando, 2004).

Tolerance to Other Herbicides

Transgenic HT-crop development is not limited to glyphosate tolerance only. Glufosinate is another effective nonselective herbicide that inhibits glutamine synthase. *Bar* gene from *Streptomyces hygroscopicus* encodes for phosphinothricine acetyl transferase (PAT), which inactivates glufosinate by acetylation. Transgenic glufosinate tolerant crops are commercially sold as “Liberty Link” by Bayer, where the herbicide is sold under trade name Liberty or Basta. This gives farmers option to rotate between two transgenic HT-crops having two different modes of action, which may delay building up of resistance to either of the herbicides. In addition, the introduction of mutated acetolactate synthase (ALS) gene from *Arabidopsis* and tobacco provides resistance to sulfonylurea group of herbicides (Haughn, Smith, Mazur, & Somerville, 1988). Introducing *oxy* gene from *Klebsiella ozaenae*, Pallett et al. (1997) developed resistance to Bromoxynil, a contact herbicide. The gene encodes for nitrilase, which breaks down bromoxynil to nontoxic 3,5-dibromo-4-hydroxybromobenzoic acid. Atrazine, another herbicide, kills plants by inhibiting electron transport in photosystem II. The Atrazine chlorohydrolase (AtzA) from *Pseudomonas* sp. strain ADP causes hydrolytic dechlorination of atrazine thereby detoxifying the herbicide. An *atrazine chlorohydrolase* gene (*atzA*) when transferred to tobacco (Wang, Chen, Xing, Hao, & Chen, 2010) and rice (Zhang et al., 2014) successfully degraded atrazine in transgenic plants providing HT.

Modification of the herbicide target site is another potential strategy for inducing HT. Mutation in indigenous *aroA* gene has been targeted for developing insensitivity to glyphosate, though the mutants have lower affinity to original substrate PEP and thus are less effective. A double mutant of class I and class II EPSP synthases introduced in maize was found to show high affinity to PEP but insensitivity to glyphosate. Similarly, posttranscriptional modification of endogenous acetyl-coA carboxylase (ACCase) in creeping bentgrass resulted in resistance to ACCase-inhibiting herbicide, sethoxydim (Heckart, Schwartz, Raymer, & Parrott, 2016). ALS is another target gene for many herbicides, as it catalyzes the first common step in the biosynthesis of branched-chain amino acids. Substitution of amino acids in ALS gene in transgenic amaranthus resulted in resistance to imazethapyr (Huang et al., 2016; Trucco, Hager, & Tranel, 2006).

New Strategies for HT

In recent days, novel transgenic approaches are also being evaluated for imparting HT in crops. One potential strategy is to introduce genes that can utilize phosphite instead of phosphatase as a source of phosphorus. Phosphite is a reduced form of P that is readily absorbed by plants but cannot be metabolized by higher plants; only certain bacteria with *Phi dehydrogenase* (*PtxD*) activity can utilize phosphite. Transgenic plant with *PtxD* serves a

dual purpose; transgenic plants will be able to survive under phosphate deficiency and also natural weed population will die due to phosphate deficiency. *Arabidopsis* and tobacco with *PtxD* required 30%–50% less phosphorus fertilizer and showed 2–10 times more biomass accumulation than weeds (López-Arredondo & Herrera-Estrella, 2012). More recently, Manna, Achary, Islam, Agrawal, and Reddy (2016) introduced *ptxD* gene from *Pseudomonas stutzeri* to rice and observed higher growth and development under phosphite fertilization.

Molecular Plant Breeding and Genomics-Assisted Crop Improvement

As transgenic strategy targets the insertion of gene from other organism, long standing battle to establish a transgenic crop due to biosafety issues and public misconception has debarred many countries from adopting transgenic crops for enhancing productivity. Even in countries promoting the cultivation of transgenic crops, a substantial period and resource of transgenic research is spent on releasing a transgenic crop cultivar, adding costs to the final product and delaying release of the cultivar. Molecular plant breeding, which is a collective term for creation, analysis, and selection of plant genetic materials using molecular markers and genomics information (Moose & Mumm, 2008), has rather been well accepted in public domain as a reliable method for crop improvement. Molecular breeding is currently an integral component of almost all the major agricultural and horticultural crop breeding programs. Transfer of transgene from one cultivar to another cultivar using transgene specific markers also comes under the purview of molecular breeding, though the products are scanned under biosafety protocols and are considered as transgenic cultivars.

DNA Markers and Marker-Assisted Selection

DNA markers or molecular markers are fragments of DNA that are used to identify variations in the genetic material (allelic/nonallelic) among individuals. A vast array of DNA markers has been developed to date, based on types of target sequences (random, repeat elements, single nucleotide, gene sequence, and cDNA), DNA fragment binding oligonucleotides, and detection methods. Botstein, White, Skolnick, and Davis (1980) developed restriction fragment length polymorphism (RFLP), the first DNA marker system by using restriction digestion of genomic DNA and detection of desired genetic fragment by probe hybridization and Southern blotting. A number of modified marker systems have been developed from RFLP, including cleaved amplified polymorphic sequences (CAPS), single strand conformation polymorphic RFLP (SSCP-RFLP), and variable number of tandem repeats (VNTRs). Various random and locus-specific marker systems have been developed and utilized for understanding diversity, population structure and

evolution, construction of genetic maps, and identification of genes and quantitative trait loci (QTL) as well as developing strategies for marker-assisted plant breeding strategies. Several marker systems such as simple sequence repeats (SSRs), single nucleotide polymorphism (SNP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSRs), sequence characterized amplified region (SCAR), diversity array technology (DArT), sequence-related amplified polymorphism (SRAP), restriction-site associated DNA (RAD), start codon targeted polymorphism (SCoT), target region amplified polymorphism (TRAP), insertion–deletion polymorphism (InDel), and functional markers like genic SSRs, resistance gene analogs (RGAs) and transcription factor–based DNA markers have been developed to date that are utilized for evolutionary studies, germplasm diversity analysis, genome mapping, and marker-assisted selection (MAS). While neutral markers like SSR, AFLP, and ISSRs are being utilized over two decades for linkage map construction, QTL identification, genetic diversity analysis, population improvement, genetic resource management, and fingerprinting of crop cultivars, new marker systems such as SNP, RAD, SCoT, SRAP, genic SSRs, and RGAs are gaining importance in genetic mapping, MAS, and evolutionary studies (Chen et al., 2014; Davey et al., 2011; Satya & Chakraborti, 2015; Wang, Xu, Song, et al., 2014).

Linkage Mapping and QTL Identification

The initial phase of molecular breeding started with the identification of anonymous DNA markers cosegregating with target traits and performing marker-based selection of traits. Mapping populations where heterozygotes are absent (recombinant inbred lines, doubled haploids) or where only two classes are present (backcross population) are more suitable for genetic mapping using dominant markers like AFLP and ISSR. However, codominant markers like SSRs and SNPs are more suitable for genetic mapping in populations containing heterozygotes. To increase genetic variation Multi-parent Advanced Generation Intercross (MAGIC) populations (Cavannagh, Morell, Mackay, & Powell, 2008) have been developed in a number of crop species including rice, wheat, maize, cotton, and jute (Sarkar et al., 2016). This population can be used for both linkage and association mapping for large-scale gene-trait analysis.

Marker systems like SNP, DArT, and RAD are currently being utilized to develop ultra-high density genetic maps. SNPs are single base-pair changes in a genomic location and can be both in coding (genic) and noncoding regions. Due to abundance in genome, SNPs are currently the most popular choice to develop ultra-high density genetic maps. DArT markers are microarray-based dominant markers that screen for SNPs and InDels (Jaccoud, Peng, Feinstein, & Kilian, 2001). Ease of screening large number of genotypes makes this marker system a popular choice for diversity

analysis, linkage mapping, and QTL identification (Akbari, Wenzl, Caig, et al., 2006; Raman, Raman, Kilian, et al., 2013). RAD sequencing (RAD-seq) involves sequencing short genomic regions surrounding restriction sites in genome and development of SNP markers by reduced representation of genome (Miller, Dunham, Amores, Cresko, & Johnson, 2007). Large number of RAD tags can be identified by varying restriction enzyme combinations for digestion.

One of the earliest RFLP-based saturated genetic maps was constructed in a backcross mapping population of *Oryza sativa* and *Oryza longistaminata*, containing 726 markers spanned over a length of 1491 cM (Causse, Fulton, Cho, et al., 1994). To map yield and yield contributing traits, many maps have been constructed further in rice using *sativa* × *rufipogon* BC mapping population. For example, Xie, Jin, Song, et al. (2008) identified seven QTLs for plant height, heading date, spikelets per panicle, grains per panicle, panicle length, spikelet density, and 1000-grain weight from BC₃F₄ NILs. In addition, intervarietal crosses have also been utilized for mapping these traits; Liu, Shao, Kovi, and Xing (2010) mapped nine QTLs for 1000-grain weight and spikelets per panicle. Using a new plant type (NPT)-based mapping population of rice, Marathi et al. (2012) also identified QTLs for days to 50% flowering, panicles per plant, flag leaf length, flag leaf width, spikelets per panicle, filled grains per panicle, percent spikelet sterility, thousand grain weight, and spikelet setting density.

A wheat consensus map consisting of 4030 RFLP, SSR, and AFLP markers was developed by Appels (2003) that spanned over 3235 cM. Another consensus map of size 2569 cM was produced by Somers, Isaac, and Edwards (2004) containing 1235 SSR markers. Cui et al. (2014) also developed a genetic map of wheat that covered 3930.7 cM and identified 22 and 12 QTLs for yield and nitrogen response, respectively. In durum wheat Maccaferri et al. (2008) identified 16 QTLs for grain yield, of which two major QTLs were on chromosome arms 2BL and 3BS. Several QTLs have been identified for resistance to rust, smut, and bunt diseases in wheat (Singh, Knox, DePauw, et al., 2016). A good number of QTLs have also been identified in wheat for several root traits including length, number, biomass, elongation and volume, nitrogen uptake and tolerance to aluminum and boron toxicity and phosphorus deficiency. The root trait QTLs are useful for selecting drought- and salt-tolerant genotypes, while the QTLs associated with high nitrogen uptake can be targeted for increasing grain yield. In barley, a consensus map of 1081 cM was developed by integrating information from three recombinant inbred lines (RILs) and two DH populations (Marcel et al., 2007) and another consensus map using seven DH populations was constructed by Wenzl et al. (2006) containing 2935 markers over 1161 cM.

A 1850-marker map was constructed in maize by Coe, Cone, McMullen, Chen, and Davis (2012) from a parental population of B73 and Mo17,

containing more than 1000 RFLP and 850 SSR markers. From a synthetic population from the same parental lines, [Liu, Niu, Gonzalez-Portilla, Zhou, et al. \(2015\)](#) developed a ultra-high density genetic map with 1,151,856 SNPs, 2916 traditional markers, and 6618 bin markers using bin map strategies. Several QTLs have been located in maize for morphological traits, yield and component characters, and disease resistance ([Khairallah, Bohn, Jiang, et al., 1998](#); [Stuber, 1995](#); [Wight, Kibite, Tinker, et al., 2006](#)). Detailed description of the QTLs detected in cereal crops can be accessed from Gramene QTL database (<http://archive.gramene.org/qlt>).

Of the oilseed crops, integrated genetic map of peanut was developed by [Qin, Feng, Chen, et al. \(2012\)](#) that contained 324 SSR markers covering 1352.1 cM. In soybean, an integrated map was developed by combining 1141 SNPs, 1041 SSRs, 709 RFLPs, and 125 other markers ([Choi, Hyten, Matukumalli, Song, & Chaky, 2007](#)). A consensus map of rapeseed (*B. napus*) has been developed by [Raman, Raman, Kilian, et al. \(2013\)](#), which places 2457 DArT markers on 1987.2 cM map length. They also identified several genes and QTLs for seed and oil quality attributes, phenological components, plant architecture, sucrose transport, male sterility, and resistance to blackleg disease and boron uptake. Another integrated map of *B. napus* contains 5764 SNP and 1603 PCR markers ([Delourme, Falentin, Fomeju, et al., 2013](#)).

Of the two major fiber crops, cotton and jute, several linkage maps have been developed in cotton. [Zhao et al. \(2012\)](#) constructed a high-density map containing 2734 SSR loci covering 3668 cM. Another cotton map contains 5521 SNP markers with a total distance of 3259.37 cM ([Zhang, Shang, Shi, Huang, et al., 2016](#)). This map was also used for the identification of 18 QTLs contributing to boll weight. In jute, [Topdar et al. \(2013\)](#) constructed first complete linkage map containing 82 mapped SSR loci and detected a total of 26 definitive QTLs for fiber yield, fiber quality, and related traits. A high-density RAD-SNP linkage map in jute (*Corchorus olitorius*) has been developed by [Kundu et al. \(2015\)](#), which contains 638 SNPs (503 RAD markers) over a length of 358.5 cM, covering 87.0% of the *C. olitorius* genome. This map was used to precisely map fiber yield and its component characters, including histological fiber content. A major QTL for histological fiber content was colocalized on linkage group 1 with QTLs for fiber yield and plant height. RAD maps have also been used for the detection of QTLs for resistance to late blight in tomato ([Chen et al., 2014](#)) and rust resistance in sunflower ([Talukder et al., 2014](#)).

Marker-Assisted Selection (MAS)/Backcross Breeding (MABB)

MABB has been most successful in transferring disease resistance, as it avoids phenotypic disease screening which is laborious and often provides inaccurate results. Markers flanking resistance genes can be used for rapid

genotyping, screening out the susceptible plants. This approach has been quite successful for transferring resistance to diseases and pests and transfer QTLs related to resistance, yield grain quality parameters. One of the most successful examples of MABB is the transfer of submergence tolerance in rice. A major locus on chromosome 9, *Sub1*, provides submergence tolerance through the regulation of ethylene- and GA-mediated response in rice by quiescence in shoot elongation. This locus has been transferred in mega varieties of rice in India and Bangladesh, where about 6.8 million ha rice area is periodically affected by submergence. The first submergence-tolerant mega variety Swarna-Sub1 was developed by MABB of Swarna, a popular Indian rice variety with 45% increase in yields over the popular varieties under 10 days submergence (Dar, de Janvry, Emerick, Raitzer, & Sadoulet, 2013; Neeraja et al., 2007). The *Sub1* locus has also been transferred in BR11, a mega rice variety from Bangladesh (Iftekharruddaula, Newaz, Salam, et al., 2011).

In contrast to rice, where examples of MABB are plenty, such breeding efforts are lacking in other cereal crops. Many resistance genes against rust, head blight, and powdery mildew disease have been mapped in wheat, but reports of successful MAS are only coming up recently. For example, stem rust resistance genes *SrTA10187* and *SrTA10171* from *Aegilops tauschii* were transferred in wheat through MAS by Olson et al. (2013). Guo, Zhang, Hou, et al. (2015) transferred *Fusarium* head blight resistance gene *Fhb7* from *Thinopyrum ponticum* into wheat and pyramided the gene with another resistance gene, *Fhb1*. In another study, Yaniv et al. (2015) attempted MAS for stripe rust resistance gene *Yr15* in hexaploid wheat from wild emmer wheat. In maize, MAS has been utilized for developing resistance to southwestern corn borer (Willcox, Khairallah, Bergvinson, et al., 2002) or selection of genotypes with low phytic acid content (Sureshkumar, Tamilkumar, Senthil, et al., 2014). A number of marker-assisted recurrent selection schemes have also been developed in maize for genetic improvement of complex traits like yield. MAS has also been effectively utilized to transfer resistance to *Ascochyta* blight and *Fusarium* wilt in chickpea (Ahmad, Mumtaz, Ghafoor, Ali, & Nisar, 2014).

MAS has also contributed to the development of disease-resistant varieties in vegetable crop species, particularly in tomato and potato. Selection for resistance to tomato leaf curl virus disease was performed using a resistance gene associated codominant SSR marker to identify 82 resistant plants from an interspecific cross-derived progeny population (Kumar, Tiwari, Datta, et al., 2014). In a North American potato breeding program, resistance to potato virus Y was identified in 19 clones of potato using linked markers RYSC3 and YES3-3B (Fulladolsa, Navarro, Kota, et al., 2015). Using a combination of conventional selection and MAS (Hanson, Lu, Wang, et al., 2016) also developed five tomato lines resistant to six different diseases.

Marker-Assisted Gene Pyramiding

Marker-assisted gene pyramiding helped the accumulation of different genes contributing to single or multiple traits in a common genetic background. Gene pyramiding in crop has mostly been targeted for elevated resistance against single pathogen or pest or to provide durable resistance to multiple pathogens/pests/stresses, particularly in rice. Examples of gene pyramiding for in other crops are limited. Marker-assisted gene pyramiding for disease resistance in rice has been targeted to impart resistance to different pathotypes of bacterial blight (Pradhan et al., 2015; Suh et al., 2013) and blast (Pinta, Toojinda, Thummabenjapone, & Sanitchon, 2013); to combine resistance to bacterial blight and blast (Ellur, Khanna, Yadav, et al., 2016; Jiang, Yang, Ali, & Mou, 2015; Narayanan et al., 2002; Yasuda, Mitsunaga, Hayashi, Koizumi, & Fujita, 2014); bacterial blight, sheath blight and blast (Singh, Singh, Singh, et al., 2012); and to provide high resistance to insects like brown planthopper (Hu et al., 2013; Liu, Chen, Liu, Dai, et al., 2016). Further, there are some examples of targeting gene pyramiding for complex traits like yield and yield components. Zong et al. (2012) pyramided eight grain yield related QTLs that showed higher panicle and spikelet size. They also proposed a marker assisted and phenotypic selection (PS) based breeding scheme to minimize F₂ population size having positive QTL effects by using marker-based selection in F₂ population itself. Pyramiding of three QTLs for grain yield under reproductive stage drought stress in rice cultivar MR219 also resulted in yield advantage over 1500 t/ha (Shamsudin et al., 2016).

Genomics-Assisted Crop Improvement

The major applications of genomics-assisted crop breeding are to develop genomic resources in target crop, including the development of genome, transcriptome, proteome and metabolome databases, the development of SSR and SNP markers linked to target genes, the mapping of genes and QTLs linked with economic traits, and finally the linking of information related to a product or a pathway in a comprehensive manner so as to identify one or few crucial target genes for genetic improvement or genetic modification (Borevitz & Ecker, 2004; Rafalski, 2002). New advances in sequencing strategies, popularly known as next generation sequencing (NGS) strategies, have greatly influenced breeding strategies of various crops by allowing genome-wide association studies (GWAS), genotyping by sequencing (GBS), and genomic estimation of breeding values (GEBV) enabling genomic selection (GS) for the identification of superior genotypes. Collectively, these new tools are being utilized intelligently for better genotyping and phenotyping in the next generation plant breeding programs (Barabaschi et al., 2016; Ray & Satya, 2014).

Genotyping by Sequencing

GBS, first described by [Elshire et al. \(2011\)](#), is an elegant NGS approach based on reduced representative sequencing for the generation of large-scale genomic information. GBS does not require previous information on the target genome and can generate large number of SNP information in a cost effective way, thus it is also useful for nonmodel species where genome information is very limiting. GBS has several applications in crop improvement including genetic diversity analysis, evolutionary and population structure analysis, linkage mapping, association analysis, and GS ([He et al., 2014](#)). GBS has been employed to construct high-density genetic map and to identify QTL clusters for grain shape and grain chalkiness and seedling salinity in rice ([Chen et al., 2016](#); [De Leon, Linscombe, & Subudhi, 2016](#)). A GBS-based map containing 28,644 GBS markers is available in wheat, which harbors three rust resistance genes (Sr58/Lr46/Yr29, Sr2/Yr30/Lr27, and Sr57/Lr34/Yr18) and 15 published QTLs for wheat rusts ([Li, Vikram, Singh, et al., 2015](#)). GBS-based maps are also available in other crops including wheat, barley, maize, chickpea, rye, perennial ryegrass, cabbage, *Medicago*, and oat ([Liu et al., 2014](#); [Poland, Brown, Sorells, & Jannick, 2012](#)). More recently, GBS-based pipelines are being developed for major crops for SNP discovery, germplasm characterization, and genetic analysis ([Melo, Bartaula, & Hale, 2016](#)).

Genome-Wide Association Studies

While linkage analysis based on segregating population requires related individuals, GWAS or whole genome association study (WGS) allows the analysis of unrelated individuals for the detection of QTLs without pedigree information. In human genetics, GWAS is particularly helpful to identify markers associated with complex diseases; this is achieved by correlating marker allele frequencies with trait variation in a population ([Stranger, Stahl, & Raj, 2011](#)). Thousand, even millions of allele frequencies are compared, for which SNP markers are considered to be the best choice. Only few SNP variants would be associated with the phenotype, which are detected by a genomic control procedure involving chi-square test corrected for a variance inflation factor. The efficiency of detecting a robust marker test association depends on several factors, including sample size, trait allele frequency, marker density, and population structure. The population diversity determines the linkage disequilibrium (LD) of the trait. To detect robust marker trait association, marker density should be higher than LD ([Brachi, Morris, & Borevitz, 2011](#)).

GWAS has been used to understand the genetics of several complex traits in plants, such as yield, flowering time, root architecture, plant height, leaf size, drought tolerance, frost tolerance, disease resistance, grain quality traits, tolerance to micronutrient deficiency, and many others traits. Grain yield in

cereals is a complex trait influenced by many contributing and associated traits. Using GWAS, [Edae, Byrne, Haley, Lopes, and Reynolds \(2014\)](#) identified a multi-trait region on chromosome 5B of wheat contributing to yield and yield components and also observed that QTLs for grain yield and harvest index are colocalized on chromosome 1BS. In another study, QTL for grain yield was identified on chromosome 5A and 6A, while QTLs for canopy temperature, chlorophyll index, biomass, and harvest index were also determined on other chromosomal regions ([Sukumaran, Dreisigacker, Lopes, Chavez, & Reynolds, 2015](#)). Flowering is another complex trait, controlled by over 100 genes. A number of GWAS have been attempted to identify marker trait association for flowering time in *Arabidopsis*, soybean, *Medicago*, and chickpea.

Resistance to diseases in plants is often under complex regulation involving the arrays of basal and specific disease resistance response. [Thoen, Olivas, Nelson, Kloth, et al. \(2016\)](#) studied association 214,000 SNPs with 30 different abiotic and biotic stresses in 350 *Arabidopsis* accessions using a multi-trait GWAS. Their results suggest that multiple SNPs are involved in broad spectrum resistance, indicated by large QTL allele substitution effects under stress combinations. Several candidate genes for stress response including transcription factors were identified for multiple disease resistance. Besides, GWAS has led to the identification of loci contributing to resistance against many diseases including northern corn leaf blight, head smut, and common rust in maize ([Ding et al., 2015; Wang et al., 2012](#)), anthracnose and angular leaf spot in *Phaseolus* ([Perseguini et al., 2016](#))

Apart from SNP, SSR markers have also been found suitable for GWAS. [Wang, Jia, Ghai, Lee, and Jia \(2015\)](#) identified 21 SSR markers associated with blast tolerance in rice and other SSR markers associated with seed weight, heading date, and plant height in a set of 151 rice accessions genotyped with 156 SSR markers. A set of 250 SSR markers was used for association mapping of 230 Indian wheat cultivars using multi-locus mixed model approach. GWAS identified seven robust marker-trait associations for plant height, hectoliter weight, hardness index, sedimentation value, and grain weight ([Jaiswal et al., 2016](#)). SSR markers have been used for the identification of QTLs contributing to resistance in soybean and *B. napus* against *Sclerotinia* stem rot ([Bastien, Sonah, & Belzile, 2014; Gyawali et al., 2016](#)).

Genomic Selection

GS, a variation of MAS, is based on whole genome marker information to derive genotype phenotype relationship. Theo Meuwissen and his colleagues developed the initial framework of GS in 2001 using linear regression, best linear unbiased prediction, and Bayesian estimation in a simulated population for estimating relationship between true breeding values and estimated breeding values (BV). Following this approach, [Bernardo and Yu \(2007\)](#) in

another simulated study showed that genome-wide selection, where BV of a trait is predicted on the basis of the markers distributed over genome, is more efficient than marker-assisted recurrent selection in plant. While practical benefits of genomic selection became evident in dairy cattle as early as in 2006, GS in crop breeding was initiated during 2009–11 in international wheat and maize breeding programs (Crossa et al., 2010; de los Campos et al., 2009). Real genetic gain up to 50% was demonstrated in maize RIL population (Massman, Jung, & Bernardo, 2013). Presently, the development of prediction models and the development of optimal training population (TP) for higher selection efficiency are major research focus of GS. Random regression best linear unbiased prediction (RR-BLUP), initially proposed by Meuwissen (2001), has remained as the method of choice for prediction; however, other methods like random forest or multiple linear regression (MLR) have been found to be sometimes more efficient, even within same study. For example, Spindel et al. (2015) observed in rice that for grain yield RR-BLUP was most efficient, while for plant height and flowering time random forest/MLR was more accurate.

In GS, a genomic estimated breeding value (GEBV) is derived for individuals in a “Breeding Population (BP)” or test population, which has been genotyped, but not phenotyped, based on a statistical model developed in another “Training Population (TP),” which has been both genotyped and phenotyped. The allelic association of marker loci with the phenotypes in the TP is used to predict the phenotypic value of individuals having similar allelic association in the BP. Using linear regression, phenotype response or breeding value can be expressed as a linear function of marker effects. When marker phenotype association is high, the accuracy of the GS is dependent on the size of the TP, heritability of the trait in TP, and the number of loci screened. Maintaining close relationship between TP and BP is very important in each GS cycle for effective genetic gain through GS in a cost effective manner. In addition, a highly structured TP is undesirable for GS, thus population structure analysis of TP is advisable before model development. Isidro et al. (2015) showed that in highly structured rice population a stratified sampling strategy is required to develop an optimal TP for GS.

Despite new developments, the application of GS has been more useful and remunerative in animal breeding compared to plant breeding, because a single individual with high phenotypic value for economic traits like milk or wool production is priced heavily. Besides, PS is more effective in plants where large-scale replicated trial is possible reducing components of environmental variability, while in animals, breeding value is often determined from relatives, which is less efficient. In addition, most of the economic traits in plant-like grain yield are under complex genetic network and are under high genotype by environment interaction. Thus the development of suitable model and the implementation of GS in plant breeding for high genetic gain are more challenging for plant breeders. Till date, a number of

GS-based breeding strategies have been devised in different crops including wheat, maize, rice, rapeseed, sugar beet, and barley, but the cost of large-scale genotyping is a limiting factor. Cost estimates for GS in rice for a single plot are roughly \$20–30 (Spindel et al., 2015), thus with the present genotyping and phenotyping costs GS is not yet economic to PS in crop plants. However, in high value crops, fruit crops, and other perennial crops where cost of phenotyping is high, GS has an economic advantage over PS.

BIOTECHNOLOGY FOR IMPROVING NUTRITIONAL QUALITY: TRANSGENIC PLANTS FOR BETTER HUMAN HEALTH

Being one of the important goals of sustainable development, providing nutritional security and improving human health are primary challenges in both poor countries deprived from basic nutrition and wealthy countries suffering from unbalanced diet. Nutritional deficiency is responsible for about 3.5 million deaths every year, mostly in developing countries, while unbalanced diet is major cause of heart- and bone-related diseases and disorders. Transgenic crop development for enriched and balanced nutrition is a prime focus for fighting the two pronged health problems, either by enriching major food crops with proteins, mineral elements, vitamins, and other essential nutrients or by modifying edible oil quality for better health. In addition, molecular breeding for enhanced nutritional quality is another effective research area for improving human health.

Golden Rice

Rice being the staple food crop for half of the global population is the major focus for the improvement in nutritional quality through genetic transformation. The most discussed transgenic rice program is the “golden rice” enriched with β -carotene (provitamin A). Development of golden rice was envisioned as a global philanthropic program to eliminate vitamin A deficiency (causing night blindness) in the rice consuming population. Ingo Potrykus and Peter Beyer are credited for the engineering of phytoene synthase gene (*PSY*) and lycopene β -cyclase gene (*LCY*) from daffodil and phytoene desaturase gene (*crtI*) from *Erwinia uredovora* in rice grain for the biosynthesis of provitamin A (Ye et al., 2000). The concentration of provitamin A in rice grain was further enhanced by using codon-optimized *crtI* gene and *PSY* gene from maize up to 37 $\mu\text{g/g}$ of rice grain. Several international efforts have been initiated to transfer the golden rice phenotype in popular *indica* and *japonica* varieties in different countries, but the release of transgenic golden rice is still tangled in various intellectual property right issues. For updated information on the status of golden rice one can visit the official website of “Golden Rice Project” (<http://goldenrice.org/>).

Biofortification of Iron in Rice

About 30% of the human population are anemic, half of which is due to iron deficiency. It is the most severe mineral nutrition deficiency, causing about 0.8 million deaths annually. Severity is high in the developing countries, where rice is a staple food crop. Biofortification of rice with iron is a desired complementary solution to combat mineral deficiency. Several approaches have been tested for increasing iron content in transgenic rice. These include the introduction of ferritin gene for endosperm specific expression, expression of iron transporter genes, engineering biosynthesis pathway of iron transporter molecules for better translocation of iron from rice stem to endosperm, enhanced production of iron phytosiderophores, or a combination of these approaches. The most widely used approach for increasing iron concentration in rice is by expressing ferritin gene. Ferritin is an iron storage protein that can bind with many iron atoms, which is readily absorbed by human body after intake. A soybean ferritin gene *SoyfeH1* was first transferred by Goto, Yoshihara, Shigemoto, Toki, and Takaiwa (1999) with a rice endosperm specific promoter, which increased iron concentration in rice endosperm by twofold. Further efforts in *japonica* and *indica* rice have resulted in 2.1–3.7-fold increase in iron concentration in polished rice (Paul, Ali, Gayen, Datta, & Datta, 2012; Vasconcelos et al., 2003). However, further overexpression of ferritin gene led to iron deficiency symptoms in rice leaves, which put a limit to increase iron concentration in rice grain using ferritin alone.

Nicotinamine is a mineral transporter molecule, which helps in translocation of iron and other minerals in plant body. A deficiency in nicotinamine production results in chlorosis, a phenotype similar to iron deficiency. Expression of a nicotinamine synthase (NAS) gene from barley under constitutive promoter resulted in threefold increase in iron concentration in shoots (Higuchi et al., 2001). Overexpression of this gene resulted in a 10-fold increase in nicotinamine and threefold increase in iron concentration in rice grain (Masuda et al., 2009). A combination of ferritin and NAS genes in mega rice cultivar IR 64 resulted in the accumulation of 15 $\mu\text{g/g}$ iron in polished seed. The transgenic rice also exhibited high zinc content (15 $\mu\text{g/g}$) as nicotinamine also transports Zn in addition to Fe (Trijatmiko, Dueñas, Tsakirpaloglou, et al., 2016).

In another approach, Mugineic acid (MA) like phytosiderophores production was targeted, which act as iron chelators for iron uptake from the soil in cereals. Rice is deficient in MA production due to the absence of crucial MA biosynthesis genes. Transgenic rice having MA biosynthesis genes from barley was showed to have 1.4 times iron concentration than the nontransgenic controls (Masuda et al., 2008). By combining ferritin gene and MA biosynthetic genes, Masuda et al. (2013) were able to increase iron accumulation 2.5–4 times in transgenic rice.

Quality Protein Maize

Development of quality protein maize (QPM) is another major crop improvement program of global importance. A recessive mutation in the gene *o2* along with modifier genes (*Om*) increases lysine in maize endosperm. Although using phenotype and marker-based selection, several QPM lines with high productivity have been produced, breeding for QPM is difficult as the phenotypes is governed by QTLs, the *o2* gene is recessive, and large-scale biochemical and molecular screening are required to monitor the phenotype and the modifier genes. Endogenous modification of *o2* and modifier genes with RNAi has led to the increase in lysine content in endosperm, but significant improvement was not noticed. Alternatively, the incorporation of lysine rice protein coding genes from other plants holds better promise. Transformation of *sb401* gene that produces a pollen-specific high lysine containing protein in *Solanum berthaultii* with maize seed-specific promoter resulted in 16.1%–54.8% increase in lysine and up to 39% increase in total protein content in maize (Yu et al., 2004). Cotransformation of *SBgLR* gene from potato with transcription factor for *ethylene responsive factor 1* gene from tomato also led to an increase of lysine content by 30% and protein content by 24% (Wang et al., 2013). Another gene *AtMAP18* from *Arabidopsis* encodes a microtubule-associated protein with high lysine content. This gene was expressed in maize with seed-specific promoter F128, resulting in significant increase in both zein and nonzein proteins in the kernel (Chang et al., 2015). Another natural lysine-rich protein coding gene, *GhLRP*, from cotton increased lysine content by 16.2%–65.0% in maize (Yue, Li, Zhao, Zhu, & Yu, 2014).

Modification of Edible Oil Quality

Improvement of edible oil quality for better human health has been a prime focus of research in oilseed crops. Increase in oleic acid in oil provides benefit by reducing blood low-density lipoprotein (LDL) cholesterol. Conversion of oleic to linoleic acid is catalyzed by a $\Delta 12$ desaturase encoded by the *FAD2-1* gene. In 1998, transgenic high oleic acid soybean line was first commercialized by DuPont Company by silencing of *FAD2-1* gene. Inactivation of $\Delta 12$ desaturase also led to an increase in oleic acid content up to 89% in *Brassica* seed oil (Stoutjesdijk, Hurlestone, Singh, & Green, 2000). In peanut, a transgenic cultivar SunOleic 95R was developed by Gorbet and Knauff (1997) that contained 30% higher oleic acid than non-transgenic control plants. An increase in stearic acid component favors solid fat formation without hydrogenation. Knutzon et al. (1992) showed that the inhibition of stearoyl-acyl-carrier protein desaturase gene through antisense RNA in *Brassica* leads to 40% increase in stearic acid level. Antisense expression of this gene in soybean also resulted in the development of high

stearic acid soybean oil (Bidney, Coughlan, Hastings, Scelonge, & Wang, 2002). However, high palmitic and stearic acid in edible oil are undesirable as these tend to increase blood cholesterol. Silencing of gene for 16:0-ACP thioesterase that catalyzes the biosynthesis of palmitic acid has resulted in lowering total saturated fatty acid in soybean, which otherwise contains high saturated fatty acid.

Linoleic acid is an ω -3 fatty acid that prevents heart-related diseases. High linoleic acid content is also desirable for nonedible oils used for paints and inks. Overexpression of *FAD3* gene in soybean resulted in 40% increase in linoleic acid content (Cahoon, 2003). Very long chain ω -3 polyunsaturated fatty acids (VLC-PUFA) like eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) are also highly desired component of edible oil for human health. VL-PUFA is synthesized in human body in very low amount from linoleic acid, thus the major requirement is met by direct consumption primarily from fish. Biosynthesis of these acids requires series of alternate elongation and desaturation, which requires stable integration and expression of several genes coding enzymes catalyzing these reactions. For example, Kinney et al. (2004) co-expressed three genes in transgenic soybean to increase VLC-PUFA content up to 35%. Wu et al. (2005) synthesized EPA in *Brassica juncea* by stepwise transformation with nine genes. An increase in γ -linolenic acid has also been observed in transgenic canola expressing 18:1 and 18:2 Δ 12 desaturases.

GENOME EDITING FOR CROP IMPROVEMENT

Genome editing refers to precise editing of genome for targeted change. The basic tool in genome editing is a “molecular scissor,” or sequence-specific nuclease that recognizes the target DNA and modifies or replaces endogenous DNA with desired sequence. Editing of genomic DNA using site-specific nucleases is a rather recently developed tool for gene modification, random or targeted mutagenesis, transgene integration, or gene knockout. The major tools for genome editing are zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/cas9 systems. In most organisms including plant system, NHEJ is the most common error repairing pathway. Being perhaps the most “hot topic” of current biotechnological tools, several reviews have been published on mechanism and scope of genome editing in biotechnology, particularly on CRISPR/Cas9 technology (Bortesi & Fischer, 2015; Hsu, Lander, & Zhang, 2014; Joung & Sander, 2013; Mei, Wang, Chen, Sun, & Ju, 2016; Yamamoto, 2015). Besides, site-specific recombinase systems like Cre/loxP, φ C31, and Flp/FRT systems are also used in genome editing.

Zinc Finger Nucleases as First Molecular Scissors

In 1996, Chandrasegaran and coworkers from John Hopkins University first constructed an artificial ZFN by combining zinc finger proteins with the nuclease domain of *FokI* endonuclease isolated from *Flavobacterium okeanokoites* (Kim, Cha, & Chandrasegaran, 1996). Due to variation in few amino acid sequences, each zinc finger has unique affinity to bind to the major groove of double-stranded DNA through array of 4–6 zinc finger domains, each recognizing 3 base pairs. The catalytic domain can induce DNA break only as a dimer, so two ZFNs should have specific orientations to induce double-stranded break. The system can be utilized to generate targeted mutations or a homologous sequence can be inserted in the cleaved region through homologous recombination. The ZFN system was first used in plant for generating very high frequency of mutations (0.2%) in *Arabidopsis* (Lloyd, Plaisier, Carroll, & Drews, 2005). The system has also been used to knockout endogenous genes like *ABA-insensitive-4*, *alcohol dehydrogenase-1* and *transparent testa-4* in *Arabidopsis*, *DICER-like* genes in soybean or deletion of endogenous gene sequences (Petolino, 2015). Using homology-directed repair, gene editing has been performed for *sulfonylurea receptor* genes in tobacco as well as *protoporphyrinogen oxidase* gene and *RPP* genes in *Arabidopsis*.

TALEN: More Efficient Molecular Scissor

TALENs are another group of genome editing tools which combine *FokI* nuclease with type III transcription activator-like effectors. These effectors have repeat variable di-residues (RVDs) of amino acids in the DNA binding domain. Specific binding occurs through RVD-nucleotide recognition. Four types of RVD exist for the four nucleotides that provide high sequence specificity for DNA binding. Apart from the structural recognition, the mechanism of action of ZFNs and TALENs is quite similar. A number of groups are working on exploiting TALEN in rice improvement; it has been used for mutagenesis in protoplast culture and inducing point mutations in *ALS* gene to develop herbicide-tolerant rice (Li, Liu, Chen, & Yang, 2016). TALEN is considered to be advantageous over ZFN in terms of specificity and efficiency, designing a delivery system for TALEN is more difficult than ZFN.

CRISPR/Cas9: The Most Efficient Scissor

The most promising genome editing tool till date is the CRISPR/Cas9 system, a natural site-specific nuclease system. In bacteria and archaea, sequence-specific breakage mechanism works as a protection mechanism against viral or subviral infection. In about 40% of the bacteria and 90% of the archaea

species, CRISPR deliver acquired immunity by targeting viral nucleic acid (Horvath & Barrangou, 2010). Within a few years of emergence of this technology, the research applications have progressed at an astounding rate ranging from microbes, human, and plants.

Application of CRISPR/Cas9 in plant genome editing was first reported in 2013 targeting *Arabidopsis*, rice, *Nicotiana benthamiana*, wheat, and sorghum through various gene transfer platforms. Jiang et al. (2013) successfully demonstrated stable expression of this system in *Arabidopsis*, tobacco, sorghum, and rice. Studies also showed that the modified genes show stable inheritance and expression for several generations. It was also observed that this system is not only efficient in point mutagenesis, but can also be targeted for large deletions in plant. Mutagenesis and gene knockout is the most common approach in resistance breeding via CRISPR/Cas9. Wang et al. (2014) developed plants resistant to powdery mildew disease in wheat by knocking out mildew-resistance loci using both TALEN and CRISPR/Cas9 technologies. Knocking out of a sucrose transporter gene *OsSWEET13* through CRISPR/Cas9 in rice provided resistance to bacterial blight disease (Zhou, Peng, Long, & Sossso, 2015). This gene is a disease susceptibility gene, recognized by the effector protein produced by the causal bacterium, *Xanthomonas oryzae* pv. *oryzae*. Mutation in the gene prohibits host–pathogen recognition, thereby preventing disease development. Recently, by targeted mutagenesis of *OsERF922* gene in rice six mutant lines have been developed, all of which exhibit resistance to blast disease at both seedling and adult plant stages (Wang et al., 2016).

The eukaryotic translation initiation factor (*eIF*) gene family provides resistance against a range of viruses, which has been a prime target for CRISPR/Cas9-mediated modification. Chandrasekaran et al. (2016) targeted this locus in cucumber and developed nontransgenic homozygous mutant plants exhibiting resistance to cucumber vein yellowing virus infection, papaya ringspot mosaic virus-W and Zucchini yellow mosaic virus. The CRISPR/Cas9 technology has also been employed to induce tolerance to abiotic stresses in plants. In maize, drought tolerance has been achieved by developing variants of *ARGOS8* gene, which codes for a protein that serves as a negative regulator of ethylene response. Genotypes having a particular variant of *ARGOS8* coupled with *GOS2* promoter exhibited elevated *ARGOS8* expression and better grain yield under drought stress at flowering stage (Shi et al., 2016). Using a different approach, by modifying a proton pump in *Arabidopsis*, Osakabe et al. (2016) have been able to enhance stomatal closure to reduce transpiration loss, increasing tolerance to drought. Several novel applications of CRISPR are also coming up; it is being used to investigate physiological processes and developmental processes as well as metabolic pathways.

MOLECULAR PHARMING FOR METABOLITE PRODUCTION IN PLANTS

Therapeutic, Nutritional, and Other Industrial Protein Production in Plant

In comparison to animal cell culture, therapeutic protein production in plant is easier, has less chance of contamination, and requires less complexity in approval of the processes and products. Compared to microbial systems, plant systems are suitable for the active form of complex protein production due to availability of posttranslational modification systems, which are absent in prokaryotes. Despite these advantages, large-scale therapeutic production using plants has not been very popular, one of the issues being low yield of therapeutic proteins under plant culture system. The other reason is the advances made in animal cell culture system, where stable approved cell lines are already available for commercial antibody production. Another popular belief was that antibodies produced in animal system like Chinese hamster ovary (CHO) cells or the murine myeloma cells lines will be more “natural” than produced in plant cells. But high risk of contamination of human pathogens with the animal cell lines in the production system has invoked commercial interest in the plant-based therapeutic production system in recent days.

Molecular farming of therapeutic proteins started in 1990 with the success of expression of human serum albumin in tobacco and potato (Sijmons et al., 1990). In 1992, the first experimental vaccine, the hepatitis B virus (HBV) surface antigen, was produced in tobacco and potato (Mason, Lam, & Arntzen, 1992). These two successes opened up new avenues in therapeutic protein production prompted further research for standardization and upgradation of the plant-based RPP systems. The range of transgenic plant-derived therapeutic proteins can be classified into three groups—antibodies, vaccines, and other proteins excluding these two groups. Production of antibodies in plant system has received most attention, because of the availability of stable general technologies for expression of the protein and culture of the host plant. But the development of antibodies in plants is more difficult as these are complex glycoproteins and need correct folding for activity. Although currently there is no plant-derived antibody in the market, several products are in the pipeline. A number of plant-derived antibodies (plantibodies) are presently in the race of getting approval for release, such as antibodies for Ebola virus, non-Hodgkin’s cell lymphoma, sexually transmitted diseases and cavities. A tobacco-derived antibody against *Streptococcus mutans* has been approved for clinical trials for fighting cavity.

Musiychuk, Stephenson, Bi, et al. (2007) developed a launch vector system by combining features of Ti plasmid and plant viral vectors for

high-level antigen production in plants. The system was tested successfully for engineering and expression of target antigens from various pathogens, including influenza virus H5N1. Using the same system, this group was also able to produce hemagglutinin and influenza virus H3N2, which provided resistance under challenge inoculation in ferrets. In another study, [Castilho et al. \(2011\)](#) produced different glycoforms of a monoclonal antibody for Ebola virus using magnICON expression system in *N. benthamiana*. [Olinger et al. \(2012\)](#) also reported the production of a mixture of three monoclonal antibodies in CHO and *N. benthamiana* cells, which protected rhesus macaques from Ebola virus infection. This was successfully applied as ZMapp, an artificial antibody comprising three chimeric monoclonal antibodies (MB-003, ZMa, and ZMb) being produced in *N. benthamiana* culture. The compound when tested on infected rhesus monkeys exhibited 100% cure ([Arntzen, 2015](#)). In 2014, ZMapp was successfully used to treat patients infected with Ebola virus and is currently under the process of further human trial for release. Another successful application for large-scale rapid plant-based vaccine development is the production of influenza virus-like particle (VLP)-based vaccines to rapidly counteract pandemics like swine flu. [D'Aoust et al. \(2010\)](#) developed a plant-based manufacturing platform for the rapid production of influenza VLPs. Similarly, another large-scale production platform of recombinant hemagglutinin proteins was developed in *N. benthamiana* from H1N1 and H5N1 strains of influenza virus ([Shoji, Chichester, Jones, Manceva, et al., 2011](#)). Different chains of murine monoclonal antibody (pHu-E16) were produced at high levels in *N. benthamiana* and lettuce plants, showing that plants have capability to assemble large and complex IgG-like tetra-valent MAb ([He et al., 2014](#)).

Apart from antibodies and vaccines, plants are also increasingly being used for nutritional and industrially important metabolite production. In 1997, avidin was reported to be commercially produced in transgenic maize. This glycoprotein is present in egg white in low quantity, but in maize, it can be produced at a 40-fold quantity ([Hood, Witcher, Maddock, et al., 1997](#)). Similarly β -glucuronidase (GUS) is also produced commercially in transgenic maize. Maize has also been used for commercial production of Trypsin ([Woodard, Mayor, Bailey, Barker, Love, et al., 2003](#)) and the purified enzyme has been marketed under the trade name TrypZean since 2002 by Sigma-Aldrich. In 2012, taliglucerase alfa, the first plant-derived biopharmaceutical protein produced was approved by the FDA for human use in the United States for the treatment of Gaucher's disease. This recombinant protein was produced by the Israeli company Protalix Biotherapeutics in carrot cell suspension cultures. Production of insulin in safflower has also been approved for Clinical trial in the Europe, which complies with good manufacturing practice (GMP), a regulatory requirement for approval as a commercial product.

Since stable culture platform production is a drawback in plant culture systems in comparison to animal culture system, lots of research efforts have

been driven toward suitable expression platform creation for the development, expression, and manufacture of recombinant proteins. The three major platforms are based on tobacco, rice, and carrot suspension culture cells. The tobacco BrightYellow2 (BY-2) line is considered to be one of the best suspension culture and transformation responsive lines, which has been used for the production of Hepatitis B surface antigen, granulocyte macrophage colony stimulating factor (GM-CSF), human Interferon, immunoglobulins, and human growth hormones (Santos, Abranches, Fischer, Sack & Holland, 2016). Similarly, japonica rice lines are more amenable to suspension culture and transformation, hence are more used for the production of human serum albumin, immunosuppressive agents, growth hormones, GM-CSF and some commercially important cosmetic products. Wuhan Healthgen Biotechnology Corporation in China has developed a recombinant protein expression platform named “OryzExpress Platform” which uses rice endosperm culture to produce a variety of vaccines, antibodies, industrial enzymes, or amino acids. ProCellEx, another platform from Protalix Biotherapeutics, is based on carrot and tobacco cell culture for the production of antibodies, enzymes, and other pharmaceuticals. It is the main platform for the production of taliglucerase alfa. In addition of these major cell lines, tomato, soybean, ginseng, and sweet potato cell lines are also being used for the production of recombinant proteins in plants.

Secondary Metabolite Production by Single Cell Culture

Long-term culture of single cells or cell aggregates in liquid media was first demonstrated by Muir in *Nicotiana* and *Tagetes* in 1953. With further improvements in culture techniques, the production of secondary metabolites became a potential application of single cell culture (Rischer et al., 2013). Liquid cultures are more suitable for automated large scale in vitro multiplication, and are therefore considered very important for commercial ventures in plant tissue culture. However, plantlets cultured in liquid medium tend to have a hyperhydric nature, known as vitrification. To overcome this, special media with less sugar and salt concentration are used. Commercially viable productions of secondary metabolites in plant cell culture include shikonin production by cell suspension cultures of *Lithospermum erythrorhizon*, ginsenosides from *Panax ginseng*, berberin production from *Coptis japonica*, rosmarinic acid production by cell cultures of *Coleus bluemii*, paclitaxel (taxol) from *Taxus* species, camptothecin from *Camptotheca acuminata*, vinblastine and vincristine from *Catharanthus roseus* and podophyllotoxin from *Podophyllum peltatum* (Köhle et al., 2002; Murthy et al., 2014; Rischer et al., 2013). Ginseng (*P. ginseng*) is used for centuries in East Asian countries as a medicinal plant. The root of ginseng contains ginsenosides and other bioactive compounds that are considered to have antiaging effect. Hairy root culture and adventitious root culture are the two major systems

for commercial cultivation of ginseng root biomass for harvesting biochemicals. Industrial-scale ginseng root culture is also growing up in Japan, China, and Korea and a number of pilot scale studies are available (Murthy et al., 2014).

Single cell culture is also an important source for rapid micropropagation, having distinct advantage over callus culture in terms of production of plantlets. Under ideal condition, about 0.1 million plantlets can be produced per year from a single source. Despite this promise, micropropagation via single cell culture has not made much commercial progress. However, it is used for the micropropagation of orchids (Lakshmanan, Loh, & Goh, 1995). It is also an important method for genetic improvement through mutagenesis, as large populations can be handled within limited space. The major drawbacks of single cell culture are cheaper alternate propagation, genetic and metabolic destabilization after long-term culture, hyperhybridization, and other difficulties in handling of liquid culture media.

FUTURE OUTLOOK

Exponential increase in human population as well as deterioration of health of our environment has driven the necessity of boosting crop productivity at much accelerated speed, which demands crop improvement to be more precise, target oriented and efficient to produce more food from limiting resources including cultivable lands, water resources, and human inputs. Our understanding of crop biology has increased considerably with the generation of huge information from genome, transcriptome, and proteome sequence studies, which on one side identifies huge number of target genes for manipulation and tells about the possible consequences of such manipulation. However, such extensive choice of genes makes selection difficult, thus the filtering of information to pinpoint crucial genes is necessary. As we have seen, many target genes were later abandoned due to potential allergen or environment risks; thus selection of transgene for genetic engineering should be of paramount importance, so as to predict response of the transgenic crops in multiple environments. The sequence and gene–protein–metabolite interaction network data can be utilized to filter the target transgenes for higher efficiency.

Gene knockout is an altogether different approach, which may bring unexpected revolution in crop improvement through accurate surgical strike on the genome. Like RNA interference, research applications of gene targeting in plants have increased tremendously in the past few years, which is a very positive sign for widespread application of these two technologies. Acceptance of these technologies by users, however, is again a great concern as observed in the case of genetic engineering; suitable policy frameworks have to develop for quick realization of the results obtained from these technologies.

For molecular breeding, in-depth understanding of crop response under changing environment is essential to identify and target specific genes and their products for bringing desirable and directional change in crop genotypes for increasing productivity. While many genes and QTLs have been mapped, information is limiting on QTL response under variable environments, which has limited the success of MAS only to particular monogenic traits or for pyramiding of genes. Maize, for example, have not benefitted much from targeted MAS for single trait, and only a few examples are there where the inbred lines have been improved through MAS for developing hybrid varieties. International maize genetic improvement programs are now gradually shifting toward GS approaches. Genetic improvement of wheat or other cereals, on the other hand, has not really exploited the potential of MAS. These crops and other essential food crops need a stronger integrated plant breeding framework implementing target-oriented crop improvement meeting SDGs and should be equipped with in-depth phenotyping and genotyping for accelerating genetic gain.

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Chapter 5

Transgenic Animal Production

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INTRODUCTION

It is admitted that domestication of some animal species occurred 10,000 years ago, and this resulted in the control of their reproduction allowing their selection. Conventional selection relies essentially on the observation of the individuals leading to the choice of the best potential genitors. Cows have become much less aggressive. Some of them have no more horns, are able to reproduce throughout the years, and they produce huge amount of milk. Some animal species such as silk worms have become unable to survive without the assistance of humans. The same is true for some pets, particularly for dogs. The domestic species of animals and plants have thus been profoundly

genetically modified (GM). These genetic modifications remained totally unknown until very recently. Most of them are still unknown.

The discovery of the heredity laws by G. Mendel in the mid of the 19th century rendered selection more efficient and more precise. Yet, conventional selection remains strictly dependent on the spontaneous natural mutations which occur randomly at a low frequency at each reproduction cycle. In order to enhance and accelerate the genetic modifications, some plants are subjected to the action of mutagenic substances. This is a currently used method, and a number of plant varieties used as feed or food were obtained in this way. In plants, hybrids obtained by the crossing of varieties allow marked improvement of food production. Two new species were even created by humans by crossing artificially two species. One of them, triticale, resulted from the crossing of rye, and wheat is cultured at a large scale and used as feed.

Such manipulations are not so easy in domesticated animals. Mutagenic compounds are also used but only to generate models for basic and medical research. Yet, mules resulting from the crossing of horse and monkey have been regularly generated for centuries. This corresponds to the transfer in a blind manner of 25,000 genes from one species into another without any particular problem. This shows that living organisms are flexible and that humans have proceeded to multiple and profound genetic modifications for their own profit without any important biosafety or ethical problems.

The discovery of DNA and the identification of individual genes have made it possible the generation of genetically engineered animals. This was demonstrated for the first time in 1980 with the birth of mice harboring foreign genes transmitted to progeny. The possible applications of this technique appeared likely after the birth of giant mice expressing exogenous growth hormone (GH) genes.

Transgenesis is thus a new potent tool for genetic selection. Transgenesis can be implemented only when relevant genes have been identified and are available. Some people claimed that it is theoretically not possible to modify important biological functions as they are all under the control of multiple genes. This is obviously not correct, and the contrary was demonstrated by the generation of the giant mice and more recently by the establishment of salmon lines showing an accelerated growth. Indeed, growth is undoubtedly under the control of multiple genes but in some species, one of them, this coding for GH, is not optimally expressed in wild animals. Thus, the addition of GH gene is sufficient in some species to accelerate growth.

Conventional selection is fundamentally limited by several parameters: (1) the natural mutations are rare and slow specially in farm animals, (2) these mutations provide animals only with different versions of endogenous genes, (3) the genes selected are generally unknown, and (4) conventional selection relies on the random rearrangement of parental chromosomes which coselect a number of genes present in the vicinity of the selected gene, leading sometimes to deleterious side effects (Fig. 5.1). On the contrary,

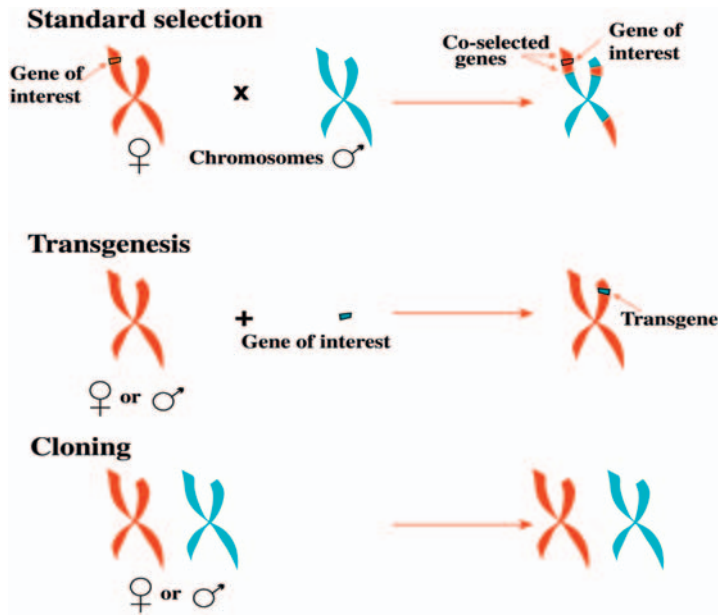


FIGURE 5.1 Impact of evolution, transgenesis, and cloning on genome modification. The classical genetic selection relies on the recombination of homologous chromosomes during gamete formation and the random distribution of parental genes to progeny. Transgenesis provides organisms in one generation with exogenous genes having known and potentially useful properties. *Reproduction by cloning prevents chromosome recombination.*

transgenesis allows the generation of GM in animals in only one generation. Moreover, the transferred foreign gene may come from various origins allowing an enhancement of biodiversity. The available techniques make it possible a fine control of transgene expression (Houdebine, 2003).

This does not mean that transgenesis is going to replace conventional selection. Indeed, the number of known key genes controlling biological functions is still very limited and may remain so for years. Lactation is a very important function for humans, but so far milk production is being improved by conventional selection but not by transgenesis. Indeed, no key genes known to enhance milk production have been identified so far. It is expected that the sequencing of farm animal genomes will allow the identification of key genes potentially utilizable for transgenesis. It is also expected that the identification of quantitative trait loci will allow a more efficient selection on the basis of correlation between the primary sequence of alleles and the biological properties of animals. This may dissuade to use the genes for transgenesis in some cases. Moreover, the beneficial genetic modifications resulting from gene transfer must be disseminated in herd using conventional breeding methods. Transgenesis is thus fundamentally not a

competitor but a complement to conventional selection. The extensive use of GM plants over the world clearly supports this concept.

A last point to consider is the fundamental specific biosafety risk of transgenesis. Living organisms are subjected to multiple natural genetic modifications. Examples are rapeseed and wheat which result from the natural crossing of two and three species, respectively. Salmonids are known to have four copies of their chromosomes. Human communities proceeded to multiple and profound genetic modifications by selection. The benefit for humans is huge with limited deleterious effects. Farmers learned to observe their animals and their plants in such a way as to keep the best of them for reproduction. It is thus generally admitted that conventional genetic selection and transgenesis are both low-risk techniques. Specific risks may rather come from the genes which are selected or transferred. In this respect, it must be considered that the genetic modifications are much better known in transgenic versus selected animals. In addition, the guidelines for the applications of transgenic animals are much stricter for transgenic than for selected animals

TECHNIQUES FOR ANIMAL GENETIC MODIFICATIONS

Transgenesis is facing to two major technical problems. One is the methods for genetic modifications proper and the other is the construction of vectors allowing a reliable expression of the transgenes. The generation of transgenic organisms implies that the foreign gene is present under a stable integrated form in the genome of the embryos in order to be transmitted to progeny. The methods to be used to transfer foreign genes are highly dependent on the available reproduction techniques in the different species. Foreign genes can be transferred to be integrated randomly or in a targeted manner according to the aim of the project. During the last two decades, a variety of techniques has thus been developed to optimize genetic modification in about 15 species including insects, fish, lower vertebrates, and mammals.

Mechanisms of Random Gene Integration

The foreign DNA artificially introduced into cells forms multimers known as concatemers including sometimes gene rearrangements and mutations. The different copies of the gene are then organized randomly in head-to-tail or in tandem when the DNA is introduced in cytoplasm or in tandem when the DNA is introduced directly in nuclei. The integration of foreign DNA fragment into a genome may occur by two different mechanisms. The most frequent process relying on heterologous recombination leads to a random integration. Targeted integration relying on homologous recombination is much less frequent.

Heterologous recombination occurs when the foreign DNA recognizes more or less similar genomic DNA sequences. Heterologous hybrids which are thus formed trigger the integration of the foreign DNA at the recognized genomic site when DNA is duplicating before cell division. In these conditions, the frequency of integration is relatively low. The different lines of transgenic animals obtained in this way are thus all different from each other. They contain variable copy number of the transgene integrated each time at a different site of the genome. The integration of the foreign gene may damage locally the host DNA. Moreover, the transgene may then be submitted to the unpredictable and unknown effects of the endogenous transcription regulatory elements located in its vicinity. The regulatory elements of the transgene may also alter the transcription of the host genes in its vicinity.

A systematic study revealed that transgenic mice heterozygous for the transgene show rare abnormalities. On the contrary, the homozygous mice appear altered in a proportion as high as 3%–10% suggesting that the random integration of the foreign DNA is relatively often mutagenic (Van Reenen et al., 2001; Van Reenen, 2009). This suggests that integration of the foreign DNA may be not fully random. Indeed, there is space for 1 million of transgenes in animal genomes and the chance of being integrated within a host gene or in its vicinity is low. The frequency of the abnormalities in transgenic mice suggests that integration occurs preferentially in regions of the genome-containing genes.

Mechanisms of Targeted Gene Integration

It is possible to target the integration of the foreign gene using homologous recombination. This mechanism is based on the perfect recognition between a chosen genome sequence and the sequence of the exogenous DNA. This recognition leads to the formation of hybrids and finally to the targeted integration of the foreign DNA (Fig. 5.2). Homologous recombination exists in all living organisms. It is naturally implemented to repair mutated genes using the other allele as a matrix, to redistribute the regions of homologous chromosomes during the formation of gametes, and to generate functional antibody genes from the genomic sequences containing the different elements of these genes. Homologous recombination is routinely used to GM bacteria and yeast. Homologous recombination is a rare event in animal cells, corresponding to about 0.1% to 1% of the heterologous recombination. It is therefore traditionally not implemented directly in early embryos but in intermediate cells further used to generate transgenic animals.

Several applications of this approach are possible: (1) the precise integration of a functional foreign gene in a chosen genomic region (gene knock in), (2) the replacement of an allele by another allele, and (3) the replacement of a gene by a nonfunctional DNA sequence leading to the inactivation of the targeted gene (gene knock out) (Fig. 5.2).

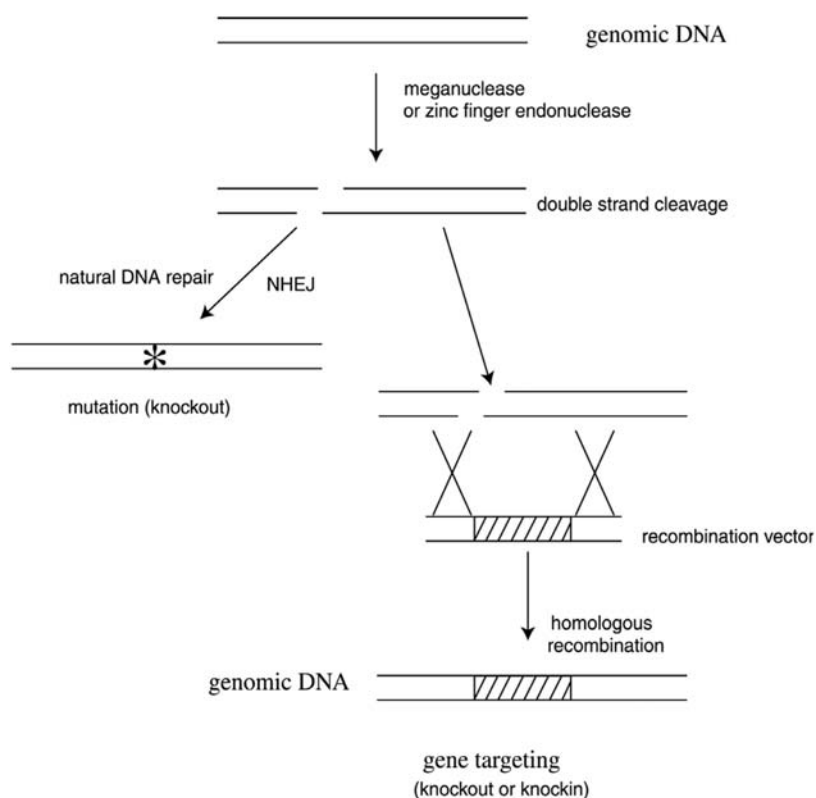


FIGURE 5.2 Gene targeting using homologous recombination. The introduction into a cell of a DNA fragment having part of its sequence similar to genomic DNA may lead to a replacement of the genomic sequence at a very low frequency. If the transferred DNA contains two sequences homologous to genomic DNA regions surrounding a foreign DNA, the homologous sequences recombine (indicated by crosses) and the foreign DNA is integrated into the genome in a targeted manner. The targeted genomic gene is interrupted and thus inactivated (knock out) by the foreign DNA. Alternatively, the foreign sequence may be a functional gene the integration of which is precisely targeted (knock in). The homologous recombination process is enhanced up to 1000-fold when both strands of genomic DNA are locally cleaved by targeted endonucleases (meganucleases, ZFN, or transcription activator-like effector nucleases (TALEN)). When the endonucleases are injected into the embryos without any recombinant vector, DNA break is repaired but often according to a random process known as NHEJ (non-homologous end joining) generating a targeted mutation and thus a knock out.

The frequency of homologous recombination is greatly increased when both DNA strands are specifically cleaved at the targeted site of the genome. To reach this goal, several endonucleases having a domain recognizing the targeted sites in the genome and another domain able to cleave DNA nonspecifically in the vicinity of the binding site may be used. These endonucleases may be meganucleases found in yeast. They cleave DNA at specific sites nonexistent in most species. Meganucleases must therefore be engineered to

target the DNA cleavage and to induce specific homologous recombination. An alternative consists of generating fully engineered fusion endonucleases, known as zinc-finger nucleases (ZFN), containing a zinc-finger region recognizing specifically the chosen genome site and a common nonspecific endonuclease, FokI. A third possibility particularly attractive is based on the generation of fusion enzymes in which the FokI domain is associated to a domain recognizing specific DNA sequences. These second domains belong to the plant TAL (transcription activator-like) effectors. The resulting endonucleases known as TAL, TAL nuclease, or TALEN can virtually be engineered to target any genomic site (Li et al., 2010; Revon et al., 2012; Nature Methods, 2012). More recently, the CRISPR-Cas9 system (Clustered Regularly Interspaced Short Palindromic Repeats) in which Cas9 is an endonuclease and CRISPR corresponds to an RNA which targets Cas9 in a chosen site of the genome (Ran et al., 2013). These engineered endonucleases may thus be used to enhance up to hundreds folds the frequency of homologous recombination and of targeted foreign gene integration. The CRISPR-Cas9 system is known as simple to use, versatile, cheap, and giving rapidly results. This system is becoming extremely popular.

Interestingly, the endonucleases make it also possible a targeted gene knock out in the absence of foreign DNA. This is achieved by a nonspecific DNA repair mechanism known as NHEJ (nonhomologous end joining). This process is known as transgenesis without transgene (Fig. 5.2).

Recent publications have shown that both targeted knock out (Rémy et al., 2010) and targeted gene integration (Meyer, Hrabé de Angelis, Wursta, & Kühn, 2010) may be obtained with good efficiency directly in embryos of mammals and fish (Woods & Schier, 2008).

Moreover, zinc finger nuclease (ZFN) can also target efficiently the integration of foreign genes bordered by the cleavage site of the ZFN into genomic sites also specifically cleaved by the ZFN (Orlando et al., 2010).

These new tools are expected to have a strong impact on transgenesis use. The engineered endonucleases can be obtained on a case-by-case basis from specialized companies or within laboratories. The engineered endonucleases are working in a broad variety of living organisms. Their specificity is generally good but off-targeting may occur without having been predicted by *in silico* analysis (Gabriel et al., 2011; Slaymaker et al., 2016). To reduce the off-targeting of NHEJ, it was proposed to use a single endonuclease and then to cleave only one DNA strand. This approach proved relevant but, as expected, the knock out frequency was reduced.

METHODS FOR GENE TRANSFER

The most efficient method to introduce foreign DNA into the genome of animal cells was traditionally to microinject the DNA into nuclei. Animal embryos are relatively rare, particularly in some species. Microinjection was therefore initially implemented to generate transgenic animals. The first

transgenic animals, mice, were obtained by microinjecting the genes into one of the nuclei (pronuclei) of 1-day embryos (Gordon, Scangos, Plotkin, Barbarosa, & Ruddle, 1980). This method could be extrapolated successfully to three other mammals (rabbits, pigs, and sheep) (Hammer et al., 1985) but it soon appeared that other methods had to be found for some other species.

Transgenesis is carried out mainly for basic research and only in a limited number of species: mammals (mice, rabbits, and rats), insects (*Drosophila*), fish (medaka and zebra fish), and worms (*Cenorhabditis elegans*). Some farm animals (rabbits, pigs, chicken, sheep, goat, and cow) are also being used for specific studies which cannot be performed easily with laboratory animals. Some farm animal productions are also expected to be improved by transgenesis in addition to classical genetic selection in the coming decades. Several different and complementary methods for gene transfer have thus been developed during the last two decades. These methods are summarized in Fig. 5.3. A comparative analysis of the methods used to generate transgenic mice has been recently published (Pease & Saunders, 2011). A large part of the advices given by the authors may be extended to other species.

DNA Transfer Into Embryos

DNA Microinjection

About 1000 copies of the gene construct contained in 1–2 pL may be injected into the pronuclei of one-cell embryos. This is possible only in mammals, as in other species, the pronuclei are not visible. The pronuclei are particularly visible in mice, rabbits, and rats. They are less visible in ruminants and not in pigs. Pig embryos are opaque as they contain lipid granules. Pig embryos must therefore be centrifuged at a moderate rate prior to microinjection. The lipid granules are concentrated at one pole of the embryo making visible the pronuclei. This treatment does not impair the survival and the development of the embryos. The microinjected one-cell embryos are then transferred to hormonally prepared recipient females or to pseudopregnant females in the rabbits.

This method requires a large number of embryos. It implies a superovulation of the females by a hormonal treatment followed by a mating with a male or by an artificial insemination. The yield of this method in mice is 1–3 transgenic for 100 microinjected and transferred embryos. Despite its drawback and the fact that it is laborious, this technique is still the most frequently used in mice and rabbits. For unknown reasons, the efficiency of DNA integration is lower in all the other mammalian species and very low in ruminants.

DNA microinjection in pronuclei gives birth to at least 30% of mice mosaic for the transgene. The transgene is then not present in all the cells of the transgenic founder and particularly not in all the gametes. This is due to the fact that the integration of the foreign DNA occurs sometimes not in the

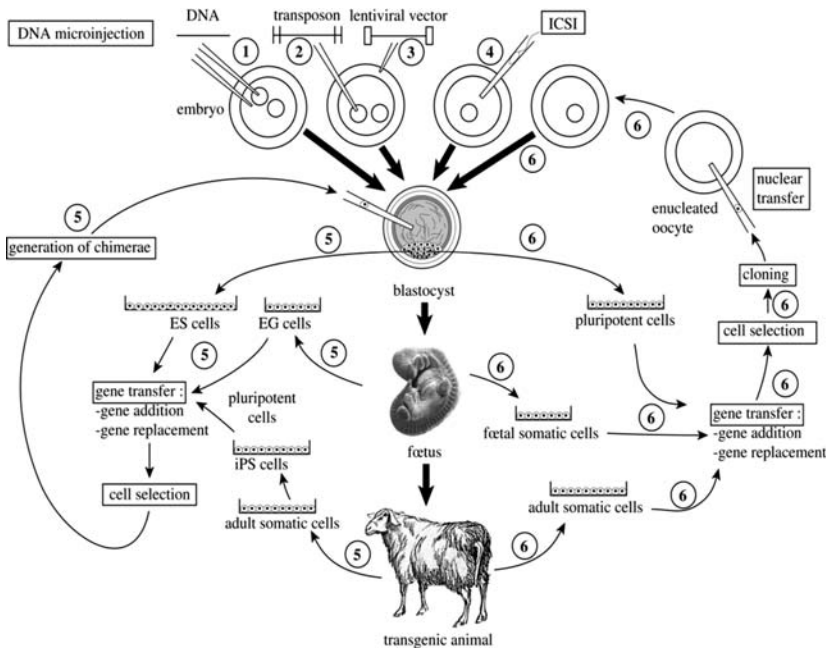


FIGURE 5.3 Different methods to generate transgenic animals: (1) DNA transfer via direct microinjection into pronucleus or cytoplasm of embryo; (2) DNA transfer via a transposon: the foreign gene is introduced in the transposon which is injected into a pronucleus; (3) DNA transfer via a lentiviral vector: the gene of interest introduced in a lentiviral vector is injected between the zona pellucida and membrane of the oocyte or the embryo; (4) DNA transfer via sperm: sperm is incubated with the foreign gene and injected into the oocyte cytoplasm for fertilization by ICSI (intracytoplasmic sperm injection); (5) DNA transfer via pluripotent or multipotent cells. The foreign gene is introduced into pluripotent cell lines (ES, embryonic stem cell lines established from early embryo or iPS induced pluripotent cells obtained after dedifferentiation of somatic cells) or into multipotent cell lines (EG, gonad cells lines established from primordial germ cells of foetal gonads). The pluripotent cells containing the foreign gene are injected into an early embryo to generate chimeric animals harboring the foreign gene DNA. The multipotent EG cells containing the foreign gene are injected into chicken embryos to generate gametes harboring the transgene. In both cases the transgene is transmitted to progeny; (6) DNA transfer via cloning: the foreign gene is transferred into a somatic cell, the nucleus of which is introduced into the cytoplasm of an enucleated oocyte to generate a transgenic clone. Methods 1, 2, 3, and 4 allow traditionally random gene addition, whereas Methods 5 and 6 allow random gene addition and targeted gene integration via homologous recombination for gene addition or gene replacement including gene knock out and knock in. The use of engineered endonucleases to cut both DNA strands makes it possible targeted gene knock in and knock out in one cell embryos.

first cell stage but later at the two- or four-cell stage. The transmission of the transgene from these founders appears not to respect the Mendel law. The low transmission rate is due to the fact that the transgene is not present in all the gametes. At the next generation, the proportion of transgenic is Mendelian (Echelard, 1997). About 1% of transgenic founders do not

transmit their transgene. This occurs when the mosaicism is very high and when the transgene is rare or nonexistent in gametes.

In nonmammalian species, the pronuclei cannot be visualized as the embryo is embedded into an abundant and opaque vitellus. High amounts of DNA (millions of copies in a few nanoliters) must then be injected into the cytoplasm of the one-cell embryos. This relatively simple technique is efficient in several fish species (Maclean, 2003) but it is highly inefficient in chicken, in *Xenopus*, in some fish, and some insects. In lower vertebrates and invertebrates, DNA microinjection into cytoplasm is followed by frequent gene rearrangements and by multiple independent integrations which often occur during the first days of embryo development in the same animals. Several reproduction cycles are then required to allow a segregation of the different transgenes, until the animals contain a single integration site.

DNA microinjection into embryos is therefore a good technique to generate transgenic animals but it is insufficient in some species. Alternative methods have been found, and they are still under study.

Use of Transposons

Transposons are short genomic DNA regions which are replicated and randomly integrated into the same genome. The integration is highly efficient, and it requires the presence at both sides of the transposed regions of repeated DNA sequences known as ITR (inverted terminal repeat) and of the action of the integrase coded by the gene present in the transposon.

The foreign genes can be introduced in vitro into the transposons devoid of the integrase gene, between the two ITRs. To be integrated efficiently, the recombinant transposons must then be microinjected into 1-day embryos with the transposon integrase prepared separately. Alternatively, the integrase gene may be added to the plasmid vector harboring the recombinant transposon sequence, but not between the ITRs. After the microinjection of the plasmid, the integrase gene is expressed inducing the integration of the DNA sequence of the gene of interest located between the ITRs but not of the other regions of the plasmid.

The foreign gene thus becomes integrated into the embryos with a yield of about 1% or more. Essentially, all the transgenic insects are being generated by using transposons as vectors. Transposons also proved efficient to generate transgenic fish, chicken, and mammals (Ding et al., 2005; Dupuy et al., 2002). The use of transposons is getting greatly improved allowing the transfer of DNA fragments as long as 120 kb with an acceptable efficiency (Moisyadi, Kaminski, & Yanagimachi, 2009; Suster, Sumiyama, & Kawakami, 2009; Sumiyama, Kawakami, & Yagita, 2010). Recently, the transposon *Sleeping Beauty* (SB1 100X) was used to generate transgenic pigs with a high efficiency (Garrels et al., 2011).

Transposons appear more and more to be particularly appropriate vectors to generate transgenic animals. The vector constructions are not particularly

difficult to obtain. They often integrate as single copy, and the gene of interest they contain are generally expressed at a high level without being submitted to silencing. This suggests that the transposons are integrated preferentially in active regions of chromatin or that the ITRs protect the transgene from silencing. Moreover, several available transposons derive from the genome of species very different of the host. This precludes the complementation and thus the uncontrolled dissemination of the endogenous recombinant transposons.

Use of Lentiviral Vectors

Retroviruses are unable to autoreplicate, and they have to be integrated stably in the genome of the cells they infected to replicate. This property of retroviruses has been implemented for the first time about 15 years ago to integrate foreign genes into cells and embryos. For this purpose, the viral genes are removed from the genome of lentiviruses and replaced by the genes of interest. Lentiviral vectors are preferred to common retroviral vectors category of retroviruses, as they have the capacity to integrate the host genome even when the cells are not replicating. Viral particles are prepared by transferring the gene construct into transcomplementing cells which have been engineered to synthesize the viral proteins. The envelope is this of vesicular stomatitis virus which binds to membrane phospholipids and has thus the capacity to direct infection of a very large number of cell types. The viral particles secreted by the cells are concentrated and microinjected between the *zona pellucida* and the membrane of the one-cell embryos (Pfeifer, 2006; Ritchie et al., 2009).

The lentiviral vectors are highly efficient, and a limited number of particles must be injected to avoid multiple and simultaneous integration. Several independent copies of the transgene are often present in the same animal. Several reproduction cycles are then necessary to obtain lines of animals harboring a single copy of the transgene.

The lentiviral vectors cannot harbor more than 8 kb of foreign DNA. They are appropriate to express genes coding for siRNAs (small interfering RNA). Moreover, lentiviral sequences are recognized as foreign DNA by cells and frequently silenced.

The preparation of the lentiviral particles requires specific protocols to be successful and to be performed in safe conditions. It is thus recommended to obtain the lentiviral particles from specialized companies or public laboratories.

Use of the PhiC31 Integrase-Mediated System

In a number of species, cells contain a specific integrase, PhiC31, capable of recombining the DNA sequences *attB* and *attP* added to vectors with similar genomic sequences with a good efficiency. This recombination integrates the

vector, and it generates *attL* and *attR* sites which are not recognized by the PhiC31 integrase. The integration of the foreign DNA is thus irreversible. This system proved efficient to generate transgenic *Drosophila* (Bateman, Lee, & Wu, 2006). This system is efficient in other species but its use is limited.

Several other recombination systems rely on the use of integrases such as Cre and Flp which recognize specific sites of about 30 nucleotides (LoxP and flippase recognition target (FRT), respectively) which must be added to the animal genome. The action mass law implies that the excision of the integrated foreign DNA is more efficient than its integration. These systems can be used as tools for targeted foreign gene integration only if the integration process generates DNA sequences unable to recombine and eject the integrated foreign DNA. This approach is known as *recombinase-mediated cassette exchange* (Baer and Bode, 2001). The LoxP and FRT systems are more often used to delete a DNA region previously bordered by the LoxP or the FRT sequences.

Use of ICSI

Using sperm as DNA carrier to generate transgenic animals was shown for the first time about two decades ago. It soon appeared that this approach was poorly reproducible and thus not utilizable. The protocol consisted in incubating the washed sperm in the presence of the foreign DNA and to use this sperm for in vivo fertilization. The methods showed some efficiency in mice, chicken, and some fish. In most cases, however, the integrated DNA was highly rearranged and no more functional. This was attributed to the action of DNase present in sperm at variable concentration. The protocol was thus abandoned.

The idea was reappraised via ICSI (intra cytoplasmic sperm injection). ICSI is an in vitro fertilization technique which consists of injecting sperm into the cytoplasm of oocytes. This technique is currently used for in vitro fertilization in humans. To transfer genes, sperm from which plasma membrane has been damaged by freezing and thawing are incubated in the presence of the gene of interest and further used for fertilization by ICSI. This method proved efficient in mice (Moreira et al., 2007; Shinoara et al., 2007) and pigs (Yong et al., 2006). Interestingly, the yield of transgenesis was often higher than with DNA microinjection, and it worked as well with short and long DNA fragments. This technique is expected to be extended to the other species in which ICSI is possible.

DNA Transfer Into Intermediate Cells

The efficiency of the genetic modification is sometimes too low to be achieved at the embryo level by the methods described above. This is

particularly the case for gene targeting based on homologous recombination. One possibility to circumvent this problem is to do the genetic modifications in cells further used to participate to the development of living organisms. To reach this goal, several cell types, pluripotent cells, multipotent cells, and somatic cells are being used.

Use of Pluripotent Cells

Pluripotent cells are those present in early embryos (morula and blastocysts). Pluripotent cells have the capacity to participate in the development of all the organs including gametes. In the best conditions, the embryonic pluripotent cells can be cultured and keep their pluripotency. The resulting lines are known as ES cells (embryonic stem cells). The ES cells can be GM, selected, and transferred into recipient early embryos at the morula or blastocyst stages. These cells participate in the development of the embryo to give birth to chimeric transgenic animals (Fig. 5.3). In these conditions, the organs of the animals, including gametes, derive from the GM cells or from the recipient embryo. Some of the offspring from these chimeric animals may thus harbor the genetic modification when they derive from the transplanted cells.

The first ES cells implemented to genetically modify animals (GMAs) (mice) were used at the end of the 1980s (Capecchi, 1989; Bronson & Smithies, 1994). The pluripotency of the ES cells was already established before their use for transgenesis. It soon appeared that it was difficult to maintain pluripotency of ES cells during the period required to obtain the GM lines. It also appeared that, for unknown reasons, functional ES cell lines could be obtained only from only a few mouse strains. Optimized conditions to use mouse ES cell have described in details (Pease & Saunders, 2011).

Mouse ES cells proved to be a potent tool to knock out genes and to create unique models to study gene functions. Considerable effort for about 15 years was made to obtain ES cells from other species and particularly from rats, pigs, rabbits, and some fish. In the best cases, the ES-like cell lines were able to participate in the generation of chimeric animals. Yet, the chimerism of the animals was low, and the genome of the cells transplanted into the recipient embryos was not transmitted to progeny. It was thus considered that the ES-like cell lines were no more pluripotent but on the way to multipotency.

This raised the question of knowing the criteria, at the molecular level, to define the pluripotency state (including in particular the capacity to transfer their genome to offspring). Several metabolic pathways characterizing pluripotency in mouse ES cells have been identified. A pharmaceutical approach aimed at founding chemical compounds able to activate the pathways of pluripotency. After about a decade, genuine rat ES cell lines were obtained allowing now gene knock out and knock in as it has been the case in mice

for more than two decades (Hamra, 2010). A similar approach was extended to a few other species namely sheep and pigs, unsuccessfully so far. The chemical compounds which proved able to activate pluripotency pathways in rat embryonic cells were nonefficient in other species. Specific studies thus appear necessary to tentatively establish functional ES cell lines in other species.

Multiple studies carried out for the last 15 years led to the identification of genes required to maintain pluripotency in mouse cell lines. Recent experiments have shown that the transfer of four and even only three of the genes normally expressed in pluripotent cells, into mouse somatic cells can induce a relatively rapid dedifferentiation of these organ cells into pluripotent cells known as iPS cells (induced pluripotent stem cells). The pluripotency of these cells was ascertained by their capacity to participate in the development of chimeric mice with a transmission of their genome to progeny (Takahashi et al., 2007; Wernig et al., 2007; Nakagawa et al., 2008; Pera & Hasegawa, 2008). Interestingly, the dedifferentiation of somatic cells into iPS cells was obtained using miRNA (microRNA) chosen for their capacity to interfere with genes, and particularly Oct4 gene, involved in pluripotency (Anokye-Danso et al., 2011).

Using a similar protocol, it was soon possible to obtain iPS cells from humans and from other mammalian species. These experiments open avenues for cell and gene therapy in humans. The validity of the concept was established in experimental animals, namely mice. The real status of iPS cells has not been yet clearly defined. Recent studies suggest that the reprogramming of the genome is less complete in iPS cells than in ES cells. Additional work appears required before using safely human iPS cells.

iPS cells might also be implemented for transgenesis particularly in species in which ES cells are not available (Fig. 5.3). The concept was validated in mice but until now iPS cells have not been used to generate transgenic farm animals.

Use of Primordial Germ Cells and Testis Stem Cells

Multipotent cells which are organ stem cells able to give rise to mature gonads and gametes have been identified in several species. These cells known as primordial germ cells (PGC) were shown to be able to form chimera when transferred to early embryos. Attempts to use PGC to generate transgenic animals were unsuccessful for years. A particular effort was made in chicken. A high level of chimerism was regularly obtained when fresh PGC was introduced into early chicken from which a large proportion of blastomeres were previously eliminated by irradiation.

Experiments carried out a few years ago showed that chicken PGC can be isolated from embryonic gonads (EGs) and cultured in conditions maintaining their multipotency and allowing the establishment of stable cell lines

known as EG cells. Foreign genes can be transferred into EG cells which can be implanted into recipient embryos and participate to gonad development. In practice, the EG cells which contain the gene of interest and a selection gene are cloned, amplified, and injected into an early embryo in which the majority of the cells have been destroyed by irradiation. This gives the best chance to the EG cells to colonize the embryo and to give birth to transgenic showing a high degree of chimerism and thus transmitting their transgene to progeny with a high yield. This approach has greatly simplified the generation of transgenic chicken (Van de Lavoie et al., 2006a,b; Han, 2009). Recent studies indicated that gene transfer into EG cells is facilitated by the use of the transposon *piggyback* vector (Yang & Kim, 2012). Interestingly also, interspecific germline transmission is possible using the chicken PGC (van de Lavoie et al., 2012).

Testicular cells which are sperm precursors can be isolated, cultured, GM, more or less differentiated in vitro, and transplanted into recipient testis to give functional sperm able to generate transgenic animals by fertilization. Alternatively, sperm cell precursors may be GM in situ using viral vectors (Han, 2009; Takehashi et al., 2007). These methods are still under study, and they are not currently used to generate transgenic animals.

Use of Cloning

The repeated failure to establish ES cell lines in species other than mice and particularly in farm animals inclined to address the problem with other tools and particularly by using cloning. This implied ideally the transfer of somatic cell nuclei harboring the genetic modifications of interest into the cytoplasm of enucleated oocytes. Indeed, the success of cloning was then possible only by transferring nuclei from fresh blastomeres. This was not compatible with transgenesis which requires gene transfer into cultured cells further used to give birth to transgenic clones. The task appeared particularly difficult as nobody had been able to clone animals from somatic cells.

In a first step, it was shown that cultured blastomeres could give birth to clones. The birth of Dolly the sheep demonstrated that the genome of somatic cells can be reprogramed after being introduced into enucleated oocytes. This generates pseudoembryos capable, with a relatively low yield, to give birth to clones of the cell donor. This technique known as SCNT (somatic cell nuclear transfer) was initially designed to improve transgenesis efficiency in farm animals. This approach is likely to be used to accelerate genetic selection but its only real application is presently transgenesis (Schnieke et al., 1997; Robl, Wang, Kasinathan, & Kuroiwa, 2007). The principle of this method is described in Fig. 5.3. Genes may be transferred into somatic cells which are then used to generate transgenic clones. This method has become the most frequently used for big farm animals as it simplifies the task of experimenters and enhances the rate of transgenic animals.

Recently published important data have shown that the cloning technique does not provoke mutations in the clones (Murphey et al., 2009). Cloning in cows and sheep gives birth to the development of a number of abnormal fetuses. This problem is less frequently encountered in pigs and goats (EFSA, 2008; Houdebine, Dinnyés, Banati, Kleiner, & Carlander, 2008).

It is now well established that the abnormalities in the development of clones is due to the incomplete reprogramming of the somatic cell genome. This is strongly correlated with the incomplete DNA demethylation and to the abnormal histone posttranslational modifications. From a veterinary point of view, the clones at 6 months of age cannot be distinguished from control animals. Moreover, it has been observed that the defects of genome reprogramming in clones are essentially absent in clone descendent obtained by natural reproduction. Cloning may thus be considered not raising particular biosafety problems for breeding and for human consumers. Traceability and surveillance over several generations are however required to confirm this conclusion. It remains that cloning is the source of suffering for clones and their mothers. It should be considered however that similar abnormalities are observed in normal animals born after embryo transfer, although at a lower frequency than in clones. It is also important to note that a limited number of transgenic genitors obtained by cloning are sufficient to establish new breeds of animals.

Cloning is presently a major technique to generate transgenic ruminants and pigs. The emerging gene transfer techniques described in this review suggest that cloning might be less necessary and used to generate transgenic farm animals in the coming decade. This is expected to reduce greatly the impact of the deleterious side effects of cloning in animals (Houdebine, 2010). Cloning rabbits has been achieved (Chesné et al., 2002) but this technique is not used for transgenesis in this species. Indeed, the cloning technique has not yet been optimized in rabbits, and the use of engineered endonucleases proved efficient to target gene addition and knock out directly in embryos (Flisikowska et al., 2011). The same is true for rat (Geurts et al., 2009).

GENE CONSTRUCTION

A problem which has never been completely solved is the reliability of transgene expression. In the early 1980s, the first experiments to generate transgenic mice revealed that transgenes were often not working as expected. In a number of cases, the expression of the transgenes was very weak and not strictly specific of the promoter associated with the foreign gene. In a few cases, it was shown that the ectopic expression of the transgenes was due to the presence of genomic enhancers in the vicinity of the integrated foreign DNA. The frequent transgene silencing was thought to be induced by the integration of the foreign genes near genomic silencers. These putative

silencers were rarely identified suggesting that the ectopic transgene expression and their silencing could be not symmetrical phenomena. It was also proved that the level of transgene expression was generally not a function of the integrated copy number. In a number of cases, the expression level appeared even lower when the number of integrated copies was higher. A striking demonstration was given by the experiment in which the human β -globin gene was bordered by two LoxP sequences and integrated in mouse genome as several copies in tandem. The transgene remained silent in these mice but was reactivated in their offspring in which the copy number was reduced to one by the action of the Cre recombinase (Garrick, Fiering, Martin, & Whitelaw, 1998). Thus, for years, only empirical gene constructions having sometimes limited efficiency were used. The strategy of researchers was and often still is to generate several lines of transgenic mice (or other species) and to keep only those in which the transgene is expressed as expected. This strategy appeared insufficient when costly large transgenic animals are to be used and when finely tuned transgene expression is needed.

After about one decade, it appeared that transgene silencing was due to chromatin position effects suggesting that the transgenes were recognized as foreign sequences by some unknown cellular mechanisms. One of the most striking observations was that a genomic DNA sequence containing the whole human β -globin gene including its promoter region allowing the gene to be expressed as expected in cultured red blood cells remained silent in transgenic mice. This discrepancy suggested that the transgene silencing occurred essentially *in vivo* and that this phenomenon could take place during the early phase of embryo development, at the period in which the genome is reprogramed. A hypothesis was also that the genomic DNA sequence contained the whole β -globin gene and some but not all the transcription regulators. A confrontation of the very low expression level in patients suffering from β -thalassemia and the structure of their DNA in the genomic β -globin gene region revealed that, in some cases, the gene and its promoter were normal but that some remote regions were missing. This suggested that these regions could be the putative regulators missing in transgenic mice. An association of these regions with the β -globin gene allowed the latter to be highly expressed in transgenic mice. The extensive study of the β -globin gene locus in several species revealed that remote regulatory elements are present on both side of the locus (Table 5.1). These elements bind transcription factors specifically present in differentiated red blood cells, and they form a transcription complex known as a hub in the vicinity of the promoter through a looping process (de Latt et al., 2008). This type of mechanism seems to be common to many if not all genes in vertebrates and similarly also in invertebrates and perhaps in plants.

These observations may explain at least in part why traditionally constructed transgenes are often poorly active and they suggest using long

TABLE 5.1 What to Do If Your Transgene Does Not Work Well?

1. Evaluate the efficiency of your construct by transfecting it into cultured cells in which the promoter of your construct is active
2. Make sure that the sequence of your construct is this you expected
3. Make sure that a part of the coding sequence of your construct is not deleted after a cryptic splicing. This can be seen by a Northern blot or by RTPCR. If so, suppress (delete or mutate) the cryptic splicing (donor and acceptor) site(s) from your construct
4. Add at least one intron preferably upstream of the cDNA to avoid NMD (see below). Choose introns having good splicing consensus sequences and splicing enhancers. The second intron of the rabbit β -globin gene is recognized as one of the good introns for transgenes
5. Make sure that the mRNA coded by the transgene is not degraded by a nonsense-mediated-decay (NMD) mechanism. This occurs when the donor splicing site of the intron located downstream of the translated region is farther than 50 nucleotides from the termination codon
6. Make sure that the 3'UTR does not contain an AU rich region with the AUUUA motif which induces an mRNA degradation in quiescent cells
7. Use short 5'UTR containing not less than 80 nucleotides and being preferably AU rich to avoid the formation of stable GC rich secondary structure. The 5'UTR must not contain initiation codons within the consensus Kozak sequence
8. Make sure that the initiation codon is within the Kozak consensus sequence GCCA/GCCAAUGG
9. Reduce the overall GC content of the construct and particularly the CpG motifs in the region preceding and following the transcription start point
10. Add one or preferably two copies in tandem of the 5'HS4 insulator from chicken β -globin locus upstream of the promoter-enhancer region and optionally after the transcription terminator
11. Use a strong transcription terminator, e.g. from rabbit or human β -globin genes or from human or bovine growth hormone genes
12. Add mRNA stabilizer such as this present in the 3'UTR of α -globin gene
13. Eliminate the sequences of the transcribed region of the construct (mainly in the 3'UTR) with may be recognized by natural miRNAs of the transgenic host
14. Use as vectors long genomic DNA fragments cloned in BAC (bacterial artificial chromosome) containing the promoter chosen to express the transgene and introduce your construct (without any promoter) or your cDNA into the BAC after the promoter, for example, after the first intron
15. In bicistronic mRNA, put preferably the IRES (internal ribosome entry site) 80 nucleotides after the termination codon of the first cistron to favor the expression of the second cistron
16. Optimize codon usage if the cDNA is not of mammal origin. This modification and others in the construct may require a complete chemical synthesis of the cDNA

genomic DNA fragments contained in BAC (bacterial artificial chromosome) vectors to promote transgene expression. A number of independent observations provide researchers with recommendations to limit the failure of transgene expression.

Thus, in practice, three strategies are presently possible to optimize transgene expression: (1) to use vectors containing as many elements as possible recognized to favor gene expression, (2) to use long genomic DNA fragments as vectors in BACs expected to contain most of the elements necessary for transgene expression and preventing their silencing, and (3) to target the integration of the gene constructs in genomic sites recognized to allow a reliable transgene expression.

Nucleotidic Composition of the Vectors

Integrated retroviral sequences and transposons are inactivated by a cytosine methylation of the CpG motifs and the local formation of condensed chromatin (heterochromatin) in which histones are deacetylated and methylated in some specific sites. Transgenes seem to be inactivated by similar mechanisms. Most of the vertebrate genes contain CpG islets in their regulatory regions which contribute to their expression. Some of the CpG motifs belong to the binding site of the transcription factor Sp1 which is present not only in the promoter region of the gene but also sometimes in the first introns. This is the case for the eF1- α gene which is highly expressed in animals but poorly as transgene (Taboit-Dameron et al., 1999). An exceedingly large number CpG motif in vectors induces transgene silencing. The replacement of some of the GC regions by AT rich regions improves transgene expression. MARs (matrix attached region) are frequently found in the vicinity of genes, and they bind locally DNA to the nuclear matrix. MARs are generally AT rich, and they have been added into vectors to tentatively improve transgene expression. This approach met variable success. The *Escherichia coli* β -galactosidase gene is rich in CpG, and it is known to be a potent transgene silencer. This silencing potency proved to be markedly reduced, as the number of CpG was diminished. The coding sequences of a transgene may thus be obtained by chemical synthesis to replace a part of the CpG-rich codons by others without modifying the sequence of the corresponding protein.

Addition of Insulators

To improve the expression of transgenes, it is possible to use large genomic DNA fragments (50–250 kb) cloned in BACs expected to contain all the regulatory elements of the gene of interest (Long & Miano, 2007).

An attractive approach consists of using BACs as vectors harboring the foreign genes. The foreign DNA sequence must be introduced in the BAC using homologous recombination in bacteria. It is important to note that the transgenes driven by BACs rarely work in an ideal fashion, if only this concept has a real meaning. Long genomic DNA fragments are expected to suppress the position effects on transgenes, which is rarely the case. Indeed, it is clear that the variegated expression which characterizes the conventional

transgenes is much less frequent in animals harboring BAC vectors. A higher proportion of animals expressing the transgenes are generally found with BAC than with plasmid vectors. Some BACs may contain all the elements providing transgenes with a complete independence of the integration site. If not, a BAC vector may still contain enough regulatory elements improving significantly transgene expression to justify its use. A more sophisticated approach could consist of using as vectors containing not all the DNA sequence of BACs but only the major elements involved in the control of gene and transgene expression. This is generally not presently possible as most of the active elements present in BACs are unknown.

An insulator activity has been found in the 5'HS4 region of the chicken β -globin locus. This element contains an insulator proper and a chromatin opener (Gaszner & Felsenfeld, 2006). The 5'HS4 element can improve the expression of a number of unrelated transgenes in mammals when added into the vectors (Taboit-Dameron et al., 1999; Giraldo, Rival-Gervier, Houdebine, & Montoliu, 2003). However, the potency of the 5'HS4 element remains generally insufficient to express transgenes in a fully satisfactory manner.

Optimization of the Transcribed Region

The optimization of vectors for the expression of transgenes has been focused initially on promoters and on transcription. It is now clear that the transcribed region of genes contains multiple signals which control mRNA translation and stability. Constructing a gene for transgenesis consists often to take DNA fragments containing unknown as well as known signals and to associate them with the risk of inactivating important mechanisms for transgene expression and to generate new unknown signals. The transcribed region of the genes and transgenes is also submitted to these rules. To avoid problems, the following precaution could be taken (Fig. 5.4).

The 5'UTR (untranslated region) of the mRNA coded by the transgene must be as poor as possible of GC sequences which can stabilize double-strand hairpin structures not favoring ribosome migration to the initiation codon. The AUG initiation codon must preferably be in the Kozak consensus sequence GCCA/GCCAUGG to optimize translation initiation. The natural 5'UTR of the gene of interest may contain sequences regulating translation. It may be then useful not to keep this region and replace it by a short (not less than 80 nucleotides) AT-rich 5'UTR region from gene known to be efficiently translated in many cell types or in the targeted cells of the animals. Some mRNAs encode proteins which are not naturally secreted. Peptide signals may be added to their cDNA.

A transgene must contain at least one intron which is required to favor the transfer of the mRNA to the cytoplasm. The first intron of many genes

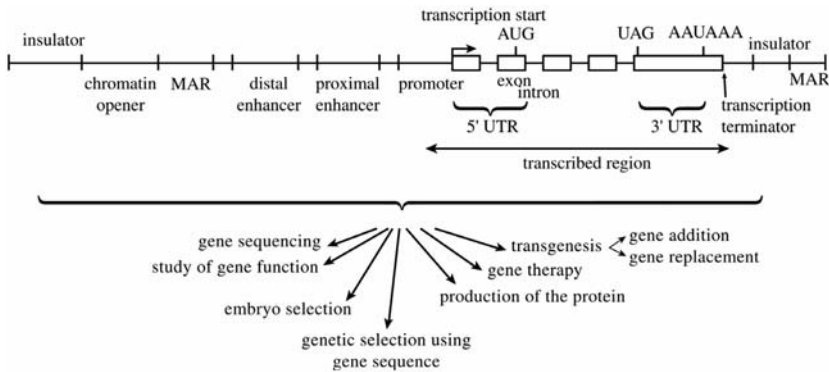


FIGURE 5.4 General animal gene structure and different uses of isolated genes. Transgenesis that includes random and targeted gene addition as well as specific gene inactivation and replacement is an essential tool for gene study and for biotechnological applications.

contains sites which bind transcription factors which may favor transgene expression. The intron splicing is dependent upon several signals comprising consensus sequences in both splicing sites (CAG GUA/GAGUA/UGGG in 5' and CAG G....GAA/G....GAA/G.... in 3'), a CU-rich region immediately upstream of the 3' splicing site and a BPS site (branched point sequence) U/CNCUGAC at about 30 nucleotides upstream of the 3' splicing site and upon splicing enhancers (Mersc, Gepperth, Suhai, & Hotz-Wagenblatt, 2008). The intron(s) must preferably be put before the coding region. If an intron is added after the translated region, the 5' splicing site must be located not more than 50 nucleotides from the termination codon to avoid the activation of the NMD (nonsense mediated decay) which degrades the mRNA (Chang, Imam, & Wilkinson, 2007).

The cDNA and other regions of the vectors may preferably be chemically synthesized. This allows reducing the number of CpG motifs, to choose the best codons, to eliminate cryptic 3' or 5' splicing sites and sequences known to prevent transcription or translation.

The 3'UTR region of a number of mRNA contains signals for mRNA translation and stability. A number of mRNAs have an AU-rich region with the AUUUA motif in their 3'UTR. These mRNAs have a short half-life controlled by the cell cycle (Beelman & Parker, 1995). The fortuitous presence of such sequences must be searched and eliminated to prevent a poor transgene expression. Some mRNAs contain translation regulators acting by the binding of proteins favoring the recycling of ribosomes by binding to the 5'UTR. CU-rich regions in the 3'UTR enhance the stability of the mRNAs, and they may be added in the vectors downstream of the cDNAs. Stabilizing sequence can be taken in the 3'UTR of the human or bovine genes and of the α -globin gene which also contain efficient transcription terminators

(Chkheidze et al., 1999). Some proteins are anchored to the plasma membrane by a GPI structure (glycophosphatidylinositol). A protein normally not anchored in this way acquires this property by adding in the 3' end of the cDNA the peptide allowing the addition of GPI. miRNAs, the role of which was recently discovered, inhibit specifically the translation of a mRNA after forming a hybrid with its 3'UTR. The presence of target sequence for a miRNA may unduly inhibit the expression of a transgene. This target sequence should then be deleted.

These suggestions are reported in a review (Houdebine, 2009a, 2009b), and they are summarized in Table 5.1.

Coexpression of Two Cistrons From the Same Vector

It is sometimes necessary to express two or even three genes in the same transgenic animals. The coinjection of several independent vectors makes it possible the generation of up to 80% of the animals harboring the two or three genes which are cointegrated at the same site. An alternative consists of using IRES (internal ribosome entry site). Such sequences exist in the 5'UTR of a number of mRNAs, the translation of which is controlled by these sequences which bind specific cellular inducible proteins. Such sequences may be added between two cistrons and allow their simultaneous translation from a single vector. The addition of the IRES 80 nucleotides after the termination codon of the first cistron may contribute to favor the expression of the second cistron (Houdebine & Attal, 1999).

Gene Inactivation

Endogenous gene inactivation is one of the most efficient approaches to study gene function. It is also a mean to suppress the expression of a biological function for some biotechnology applications. The classical use of ES cells with a knockout of individual genes allowed the inactivation of a number of mouse genes. ES cell lines with knock out genes are available to be transferred into recipient embryos and establish experimental mouse lines (Rao, 2007).

The use of engineered endonucleases, namely ZFN, TALEN, and CRISPR-Cas9, depicted above has greatly facilitated gene knock out. These tools appeared soon after the discovery of siRNAs which inhibit gene expression at the mRNA level. This process is known as knock down. The utilization of siRNAs appeared less easy and potent than this of endonucleases. Yet, siRNAs remain a valuable tool for rapid inhibition of specific genes in cultured cells. They also are appropriate to inhibit the expression of exogenous genes, namely viral genes, even if the inhibition of the targeted genes is usually not complete. SiRNAs expressed in transgenic animals are expected to protect the animals against viral infection. The success of this approach in

plants strongly supports the idea that the genetic vaccination by the use of siRNA transgenes will become a reality in laboratories and breeding.

Long double-strand RNAs present in cells are randomly cut into 19–21 nucleotide fragments known as siRNA. One of the two strands of the siRNA is kept and targeted to an mRNA having a complementary sequence. This induces the degradation of the mRNA. In practice, a synthetic gene containing the targeted 19–21 nucleotide sequence followed a short random sequence, and by the targeted sequence in the opposite orientation is linked to a promoter acting with RNA polymerase III (usually U6 or H1 gene promoters). The RNAs synthesized by such vectors form a 19–21 nucleotide double-strand RNA known as shRNAs (short hairpin RNA) are processed in cells to generate active siRNAs. An appropriate expression of siRNA genes in transgenic animals can be obtained when they are introduced into lentiviral vectors (Tiscornia, Singer, Ikawa, & Verma, 2003).

The recent discovery of the role of miRNAs has increased the possibility to use interfering RNAs. MiRNAs are encoded by short genes expressed under the control of RNA polymerase II promoters. Their primary products are transformed into siRNAs. The mature miRNAs which are fully complementary to the targeted mRNA induce a degradation of this mRNA. The miRNAs which are only partially complementary to the targeted mRNA and which recognize a sequence located in the 3'UTR (3'untranslated region) of the mRNA inhibit translation of this mRNA without inducing its degradation.

The application of the siRNA approach raises specific problems in animals. Long double-strand RNAs induce interferons and some unspecific immune reactions (Sioud, 2006). On the other hand, siRNAs are not auto amplified in higher animals, and this reduces their potency. Vectors to express miRNA gene are available but simple shRNA genes are also easily expressed in transgenic animals using conventional vectors. Moreover, siRNAs may off-target mRNAs and generate deleterious side effects.

Several programs based on empirical data indicate the putative optimal shRNA sequences use to allow the preferential use of the siRNA strand complementary to the mRNA (Jinek & Doudna, 2009). A very important point is to choose a target region of the mRNA which is not in double-strand structure and thus accessible to the siRNA. Banks of shRNA genes in lentiviral vectors are available for the mRNAs of different species. It remains that most of the siRNAs do not inhibit the targeted gene to more than 70%–80% which may be insufficient to obtain some relevant animal models. It is tempting to use vectors expressing the shRNA genes at a relatively high level. This may lead to no important increase of the inhibition and to a higher off-targeting which may be detrimental for the animals or even lethal (Sioud, 2006). In fact, it seems that a well-targeted siRNA can be highly active even at a low concentration. It appears therefore of paramount importance to select the shRNA capable of inhibiting strongly the targeted mRNA even at a low concentration in cell systems before generating transgenic animals.

Another possibility to inhibit the specific expression of a gene is to use decoys. This may be proteins, RNA, or else. A transdominant negative mutant of insulin receptor overexpressed in transgenic mice and playing the role of decoy for the hormone led to the generation of a new model for diabetes study (Chang, Benecke, Le Marchand-Brustel, Lawitts, & Moller, 1994). In transgenic chicken, the overexpression of a mutant RNA of influenza acted as a decoy preventing the formation of viral particles and leading to the generation of chicken resistant to the virus (Lyall et al., 2011). The major difficulty with this approach is to design decoys having a potent and a specific action.

Control of Transgenes by Exogenous Inducers

The vectors described above and used to express transgenes contain promoters which are naturally active in the cells of the transgenic animals. This implies that the transgenes are regulated by the natural inducers of the host genes. The induction of a transgene may then be coincident with the unwanted stimulation of a number of host genes. Artificial promoters containing regulatory elements from both animal genes and bacterial genes have been designed. The resulting promoters are active in animal cells but controlled by substances active in bacteria but not in animals. The most popular system is based on the use of the bacterial tetracycline repressor gene. In practice, the transgene becomes reversibly activated only when tetracycline (or doxycycline) is administered to the animals. A reduction of the basal expression of the transgene in the absence of the inducer may be obtained by using a repressor gene which is activated in the absence of the inducer and inactivated in its presence.

A number of similar systems are available and currently used in transgenic animals with a good success (Malphettes & Fussenegger, 2006). These tools offer the possibility to express a transgene precisely in a given cell type and at a given moment.

Gene Deletion

Conventional homologous recombination makes it possible gene deletion known as knock out. Another possibility consists of using the Cre–LoxP or Flp–FRT systems (Fig. 5.5). A LoxP sequence must first be added on both ends of the fragment to delete. The presence of the Cre recombinase will then recombine the two LoxP sites leading to a deletion of the DNA fragment located between the LoxP sequences. This makes it possible the elimination of a selection gene. The same approach allows the specific and controlled deletion of an inhibitory DNA region leading to the activation of the gene located in its vicinity. The Cre recombinase may be synthesized by the corresponding gene under the direction of a cell-specific

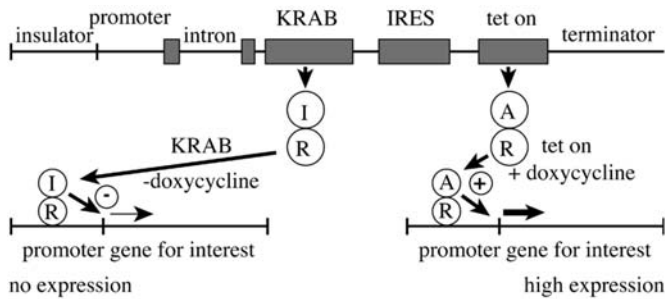


FIGURE 5.5 Transgene expression may be controlled by an exogenous inducer. In the absence of the inducer (doxycycline) the transcription enhancer (Tet on) is not bound to DNA and it does not stimulate transcription, whereas the transcription inhibitor (KRAB) is bound to DNA and reduces the background expression of the gene of interest. In the presence of doxycycline the reverse is true and the gene of interest is activated.

promoter including promoters under the control of doxycycline. Another level of control can be obtained by using an engineered Cre recombinase that becomes reversibly active in the presence of an estrogen analog, 4-hydroxy tamoxifen (Metzger & Chambon, 2001). This offers the advantage of having the active Cre recombinase for short periods of time. This prevents the nonspecific action of the Cre recombinase which can recognize cryptic sites in the host genome and induce illegitimate recombination damaging the host DNA. This tool is appropriate to delete genes for resistance to antibiotics.

USE OF TRANSGENIC ANIMALS

It has become possible to sequence the complete genome of a number of species and even of individuals. This provides researchers with a very high number of genes and alleles. The different applications of gene sequencing are summarized in Fig. 5.6. Identifying genes is a reductionist approach, and transgenesis is in some way the opposite. Indeed, the transfer of isolated genes back to animals put the gene again within its natural complexity. Moreover, transgenesis offers new application in the medicine and the breeding fields.

Basic and Medical Research

Transgenesis including gene addition and gene inactivation is a key tool to study the mechanisms which control gene expression. Using allele replacement and gene knock out is also a unique approach to identify the role of the genes in the mechanisms which govern the different biological functions. More than 90% of the transgenic animals are generated for basic studies.

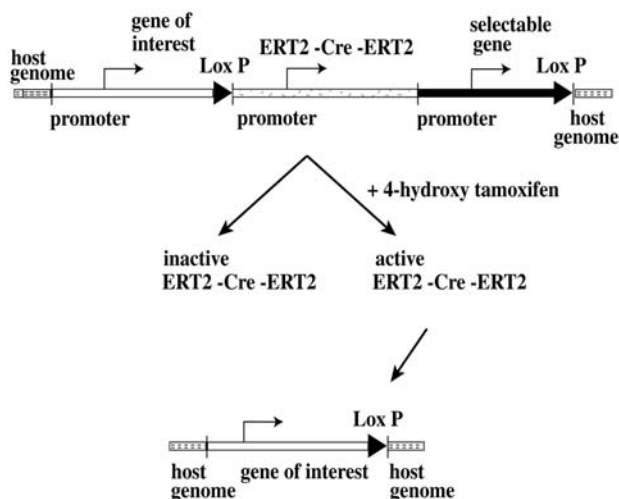


FIGURE 5.6 Activation of Cre recombinase and selectable gene elimination by 4-hydroxy tamoxifen. The Cre recombinase gene expression may be under the control of the Tet on system, itself under the control of a cell-specific promoter. The fusion protein Cre recombinase-mutated estrogen receptor is active only in the presence of 4-hydroxy tamoxifen. The elimination of the DNA region bordered by LoxP sequences is thus sharply controlled. The selection gene and the Cre recombinase may thus be eliminated from transgenic animals at any stage of their life.

Transgenic animals are then essentially models to study normal and pathogenic situations as well as pathogens themselves. A number of transgenic mimic human or animal diseases or on the contrary show resistance to diseases. Initially, essentially mice were used as models; since they are mammals, their reproduction is fast and cheap, genetic modifications are relatively easy, and several strains of mice with well-established genetic status are available. Several technical progresses depicted above make it possible more and more the use of other species. This is particularly the case for pigs (Prather, Shen, & Dai, 2008) and rabbits (Bösze & Houdebine, 2006; Rabbit Biotechnology, 2009) which are closer to humans than rodents. Rat genetic modifications have become much easier, and this species is more and more used particularly for some pathology as hypertension. Ruminants and particularly bovine are expected to be more and more implemented (Lewin, 2009).

Nonmammals species such as an insect (*Drosophila*), a worm (*C. elegans*), and a fish (zebra fish) are also used as models for studies on development.

Adaptation of Pig Organs for Transplantation to Humans

More and more organs are needed for patients, and the number of available organs is increasing more slowly. Some pig organs are expected to be an acceptable substitute. Pigs have been retained as being not too close and not

too far from humans. The techniques for breeding this species in pathogen-free conditions are available. The major problem to solve is the control of the rejection mechanisms which are very strong between different species. Genes coding for pig antigens and for endogenous retroviral sequences are being inactivated. In the same time, genes expected to inhibit locally the rejection mechanisms are added to pigs. Pig genome contains a number of integrated copies of a retrovirus which could contaminate patients to whom pig organ was grafted. In a single experiment, 62 copies of the virus were inactivated using CRISPR-Cas9 (Servick, 2015). It is expected that some neurones from the transgenic pigs secreting dopamine might be transferred to patients suffering from Parkinson disease. Pig heart and kidney grafting to humans might follow (Ayares, 2009; Petersen, Carnwath, & Niemann, 2009).

Production of Pharmaceutical Proteins by Transgenic Animals

The idea to produce recombinant pharmaceutical in milk or egg white received support in 1986, when it was proved that active human plasminogen activator was secreted in mouse milk. Several companies were then created during the following years. Several proteins have been produced by these companies, and in 2012, only two of them have received agreement from EMA (European Medicines Agency) and FDA (Food and Drug Administration) to be put on the market. Some of the early projects were not successful for various reasons. It remains that the techniques, construction of efficient vectors and improvement of the generation of transgenic animals, got improved. The proteins produced in milk and egg white are essentially as active and safe as those obtained from cultured animal cells (Houdebine, 2009a, 2009b). Guidelines for the production of proteins from transgenic animals have been defined in the EU and the United States. There is thus no technical reason not to use these bioreactors. Yet, the pharmaceutical companies are reluctant to adopt bioreactors. The increasing use of small units for the production of proteins by cultured cells rather than big fermenters offers a better flexibility at a lower cost. It is conceivable that companies may suffer from the bad image of GMOs in public opinion. Another point to consider is that companies using cultured cells to produce some pharmaceutical proteins are making substantial benefit that could be reduced when the bioreactor will be more extensively used. Indeed, the cost of a given protein prepared in milk is sevenfold lower than the same protein produced by cultured CHO cells (Chinese hamster ovary).

A project aiming at producing human polyclonal antibodies from transgenic animals is expected to have a significant impact on human health. It is known that during natural and artificial vaccination, polyclonal antibodies against the antigen(s) are produced. They generally constitute a better protection than monoclonal antibodies. It is not presently possible to obtain human

polyclonal antibodies for treating a large number of patients. The project includes the inactivation antibody genes in animals followed by the transfer to these animals of the corresponding human genes. After a vaccination, these animals contain in their blood a large quantity of only human polyclonal antibodies. These antibodies can be purified from the blood of the animals and used in humans to block pathogen infections, to inactivate some human functions as inflammation, to induce the death of tumor cells, etc., without being rejected by the patients (Echelard, 2009; Houdebine, 2011). Administration of human polyclonal antibodies from immunized cows improved health of patients suffering from Ebola disease. This project is being developed in cows, rabbits, pigs, and chicken.

Improvement of Animal Production

Transgenesis may be considered a new technique of animal selection. Transgenesis is thus expected to solve some of the pending problems in cases genetic selection failed. It may also be a more rapid and more precise to solve new problems. It is therefore not surprising to observe that the projects in course involving GMAs are essentially in the fields of traditional breeding problems (Wall, Laible, Maga, Seidel, & Whitelaw, 2009).

The major fields in question are the followings: (1) Growth was likely one of the major dominating problems for our ancestors. It is striking to see that a number of domestic plants and animals produce much more than their wild counterpart. Growth is still to be improved in some species. The case of fish is described further. (2) The struggle against diseases remains a major question. Obtaining animals resistant to diseases offers several advantages: less loss of animals and benefit, better animal welfare, less use of pharmaceutical products, and less transmission of diseases from animals to humans. (3) Optimization of meat, milk, and egg composition. (4) Reduction of pollution.

The data reported in Tables 5.2 and 5.3 summarize the most important projects in course aiming at improving animal production via transgenesis. A brief comment on these projects may be helpful to evaluate the expected impact of transgenic on food production.

Two projects aim at enhancing the amount of omega-3-rich lipids in milk and meat (Lai et al., 2006; Saeki et al., 2004; Wu et al., 2012). The consumers might improve their health by enhancing their consumption of omega-3-rich lipids. The oil from GM plants and particularly soybean containing a high proportion of omega-3-rich lipids will be available in the coming years. It is not known if the two transgenic animal projects will bring a significant advantage over soybean oil.

Monogastric animals and particularly pigs eat phytic acid from plant products, and they are unable to digest this natural compound. The phosphate contained in phytic acid is released by soil bacteria generating pollution.

TABLE 5.2 List of Projects Involving Transgenic Animals Using Conventional Techniques

Transgenic Traits	Genes	Constructs	Gene-Transfer Methods	Species	References
Production of pharmaceutical proteins	Various genes	Milk protein genes or ovalbumin promoters	Microinjection, cloning, PGC	Rabbits, sheep, goats, pigs, cows	Houdebine (2009a, 2009b)
Production of human polyclonal antibodies	Human immunoglobulin genes	Human genomic DNA fragments	Microinjection, cloning, PGC	Rabbits, chicken, pigs, cows	Echelard (2009)
Animal organs for human transplantation	Various gene addition and inactivation	Various	Microinjection, cloning	Pigs	Ayares (2009) , Niemann et al. (2011)
Increased level of poly-unsaturated fatty acids in pork	Desaturase (from spinach)	maP ₂ -FAD ₂	Microinjection	Pig	Saeki et al. (2004)
Increased level of poly-unsaturated fatty acids in pork	Desaturase (from <i>C. elegans</i>)	CAGGS-hfat-1	Somatic cloning	Pig	Lai et al. (2006)
Phosphate metabolism	Phytase	PSP-APPA	Microinjection	Pig	Golovan et al. (2001)
Milk composition (lactose increase)	α-Lactalbumin	Bovine α-lactalbumin	Microinjection	Pig	Wheeler et al. (2001)
Resistance to prion diseases	Prion protein (PrP)	Targeting vector KO	Somatic cloning	Sheep, cow	Denning et al. (2001)

(Continued)

TABLE 5.2 (Continued)

Transgenic Traits	Genes	Constructs	Gene-Transfer Methods	Species	References
Milk composition Increase of proteins	β -casein, κ -casein	Genomic fragments	Somatic cloning	Cattle	Brophy et al. (2003)
Milk composition antibacteria	Human lactoferrin	α -Slcasein-hLF Genomic DNA fragment	Microinjection cloning	Cattle	Platenburg et al. (1994) , Yang et al. (2008)
Mastitis resistance (<i>Staphylococcus aureus</i>)	Lysostaphin	β -Lactoglobulin-lysostaphin	Somatic cloning	Cattle	Wall et al. (2005)
Milk composition antibacteria	Human lysozyme	α -Slcasein-lysozyme	Microinjection	Goat	Brundige et al. (2010)
Accelerated growth	Growth hormone (GH)	Various promoters-GH	Microinjection	Salmon, carp, tilapia	Devlin et al. (2009)
Resistance to bacteria	Cecropin B	Fish promoter-cecropin B	Microinjection	Cat fish	Dunham (2009)
Avian influenza resistance	Antivirus RNA	Retroviral vectors	Infection	Chicken	Lyall et al. (2011)

TABLE 5.3 List of the Projects Involving Transgenic Animals Using Endonucleases

Transgenic Traits	Genes	Constructs	Gene-Transfer Methods	Species	References
Dehorning	Polled	CRISPR-Cas9	Microinjection	Cows	Regalado (2014)
Virus	PRRS	CRISPR-Cas9	Microinjection	Pig	Whitworth et al. (2016)
Virus	Resistance African fever TALEN	Microinjection	Pig	Pig	Lillico et al. (2013)
Virus	PERV Pol	CRISPR-Cas9	Microinjection	Pig	Yang et al. (2015)

Transgenic pigs expressing a bacterial phytase in their saliva release up to 70% less phosphate in environment ([Golovan et al., 2001](#); [Forsberg et al., 2003](#)). According to studies carried out in Canada, these animals raise no health and environment problem. Their use in breeding may not be close as the Canadian public opinion is reluctant to adopt these animals as a source of food. Moreover, some alternative approaches based on the adaptation of feed showed a significant diminution of phosphate release by conventional pigs.

Sows are producing more and more piglets, and their milk tends to be insufficient to feed them. A project which started years ago demonstrated that pig milk supplemented by exogenous proteins (bovine alpha-lactalbumin and pig IGF1) allowed a better survival of the piglets ([Wheeler, Bleck, & Donovan, 2001](#)). The real impact in breeding is not clear yet. The question remains this of knowing if it would be more reasonable to limit the number of piglets per sow.

Cows resistant to mad cow disease have been obtained by knocking out the PrP gene ([Denning et al., 2001](#)). This new genetic trait might be transferred to herds. This has not been done so far for two reasons. One is that these animals were generated mainly to obtain pharmaceutical proteins (polyclonal antibodies from blood and other proteins from milk) not contaminated by prions. The second reason is that conventional eradication proved efficient, and a very small proportion of cows still suffering from the mad cow disease results from new primary infection.

Transgenic cows in which the proportion of some milk proteins, namely of caseins, has been modified have been generated some years ago ([Brophy et al., 2003](#)). This pioneer experiment aimed at better adapting milk to the

demand of the dairy industry. This approach is obviously more efficient than conventional selection.

One of the major problems of lactating cows is mammary infections which are more and more numerous as milk production per animal increases. *Staphylococcus aureus* is the major pathogen responsible for mastitis. The secretion of a bacterial protein (from *E. coli*), lysostaphin, reduces markedly mammary infections in lactating cows (Wall et al., 2005). The cows are healthy but it remains to validate the milk from these animals. Indeed, to be efficient, lysostaphin must be present at a concentration similar to this of some of the natural milk proteins. Lysostaphin is likely not toxic but it is expected to exert an effect on the intestine flora of consumers. This impact which might be positive as a whole must be studied in depth.

Two human proteins having potent and broad antibacterial actions are present in the milk of transgenic cows and goat. These two proteins are present in many human organs and in milk. It happens that these proteins are abundant in human milk but not in cow milk. These two recombinant proteins are lactoferrin (rhLf) (Platenburg et al., 1994; Yang et al., 2008) and lysozyme (rhLys) (Cui et al., 2015). The administration of the milk from these animals to piglets modified significantly their intestine flora with a reduction of pathogenic bacteria and a better status of intestine microvilli. The milk of the transgenic cows and goats thus mimic human milk effects. This suggests that the milk of the transgenic animals could be given to consumers to prevent infection in case of epidemics or to improve intestine flora in some patients. Alternatively, tablets enriched in rhLf and/or rhLys as well as the purified proteins could be given to consumers. Likely, these two proteins should not be treated as medicaments in term of biosafety. If so, the agreement to be put on the market should not be too difficult. It remains to know if the rhLf is better than the bovine Lf already on the market and if the production in milk is competing with the other production systems (yeast, plants, ...) under study.

The production of salmon with accelerated growth is presently an emblematic project (Van Eenennaam & Muir, 2011). Indeed, it is known that salmon grow throughout their life and that the injection of salmon GH stimulated markedly their growth. It is impossible to inject repeatedly GH into small salmon for months, and it is known that salmon GH given orally to the fish has no effect. Transgenesis was thus the most appropriate system to develop salmon with accelerated growth. Several laboratories and essentially a company retained this project. The salmon GH has been fused to a promoter being active during the cold season. This led to the presence of the hormone in blood at a physiological concentration throughout the year instead of only during the warm season. It is well established that the GM salmon grow at least twice faster than the control and that they eat less feed with a reduced pollution and at a lower cost. The salmon are healthy and devoid of toxicity for consumers. The physiology of the transgenic salmon

is very close to this of the salmonns obtained by conventional selection showing also an accelerated growth (Devlin, Sakhrani, Tymchuk, Rise, & Goh, 2009). It should be noted that it took 20 years to obtain the salmonns by genetic selection and one generation via transgenesis. The major problem which seems close to be solved is this of a possible unintended dissemination of the transgenic salmonns in oceans. The transgenic salmonns would probably not have any evolutionary advantage over their wild counterpart. Indeed, the transgenic need more feed per day, and this obliges them to hunt more intensively for preys with enhanced risk to be themselves the preys (Devlin, D'Andrade, Uh, & Biagi, 2004).

Yet, the regulation agencies rejected the project as long as protocols preventing the escape of salmonns in the sea are not applied. This is now the case. The larvae (smolt) will be developed in New Found Land, and the growth phase is planned to occur in tanks of fresh water in Panama where the temperature of the sea is too elevated for the survival of salmonns. Moreover, physical barriers will be established to prevent escaping, and only females sterilized by triploidy will be grown. The protocol thus seems sound. The expected success of the GH salmonns depends of the price reduction for consumers. The demand of consumers for fish is growing. Aquaculture is in rapid development, whereas fishing is stalling. Transgenesis appears a relevant way to favor aquaculture. The success or the failure of the GH salmon project is expected to have a significant impact on other projects like this depicted in the next paragraph as well as those aiming at accelerating growth of other fish like tilapia, carp . . . which represent an important source of proteins in some countries. The GH salmonns finally received agreement from US FDA and from Canada (Ledford, 2015).

Opponents to salmon breeding claim that several kilograms of fresh fish are needed to produce 1 kg of salmon and that this is unacceptable. This reasoning seems strange as the wild salmonns need at least the same amount of fish to grow, and probably more, they spend more energy than the bred salmonns to get their feed.

Bred fish are often maintained in cages or tanks being connected to wild water, at a relatively high density. Fish are thus suffering from bacteria and virus infection. This causes loss, and it obliges to use antibiotics which are rejected in environment. It is admitted that an optimization of the aquaculture facilities and protocol would reduce these pathology problems. Yet, vaccinations against pathogens including genetic vaccinations are needed. Catfish expressing a gene coding for a cecropin A (a natural peptide having an antibacteria activity) shows a better resistance to bacteria leading to a loss diminution (Dunham, 2009).

The tools potentially used to protect animals against infections are numerous. Peptides having antibacteria activity known as defensins or bacteriocins are very abundant and a number of them could be used in transgenic animals.

An example of decoy use is mice and pigs overexpressing a gene coding for the soluble domain of the pseudorabies virus receptor. The virus is trapped by the soluble receptor, and the transgenic animals are protected from the infection (Ono et al., 2004). A protection against the same virus was obtained in mice expressing siRNAs targeted to the immediate early gene of the virus (Daniel-Carlier et al., 2013).

Chicken overexpressing a decoy RNA are protected against the infection by the influenza virus (Lyall et al., 2011). This work is considered as a perfect example of a good biotechnology project for animal production (Enserink, 2011). This project is under additional study to improve the protocol.

The elimination of mosquitos is a way to prevent the dissemination of some diseases. Several approaches are under study. Some GM mosquitoes have conditional sterility or death. The use of gene drive favors the transfer of transgenes in all the individuals of a population. This is expected to be a potent tool to eliminate or inactivate mosquitos but without an easy control of this dissemination (Esvelt, 2016).

ACCEPTABILITY OF TRANSGENIC ANIMALS

The acceptability of GM plants is variable according to countries. This suggests that GMAs will not be accepted easily (Vàzquez-Salat, Salter, & Houdebine, 2012; Vàzquez-Salat, Salter, & Smets, 2012). GM plants are well accepted in America but not in the EU. It seems that GMAs could be less easily accepted than GM plants on both sides of the Atlantic. The problem of animal welfare seems at the center of the problem. From an ethical point of view, GM plants are more accepted as the welfare problem is regarded as limited by essence for plants. On the contrary, the environmental problems are perceived as more crucial for plants than for animals not including those which swim and fly and are thus able to disseminate in vast uncontrolled areas. The suffering of GMAs is often overestimated. The methods used to generate GMAs are invasive but limited to a small number of animals (Van Reenen et al., 2001; Van Reenen, 2009) applied according to strict guidelines. The GMAs resistant to diseases are expected to suffer less than their conventional counterpart. In general, it is not surprising that the medical applications, including the use of transgenic models to study human diseases, are better accepted than the preparation of food which appears more dispensable for developed societies. The acceptability of the GMAs takes into account the specificity of the different countries toward some animal species.

In order to prepare public opinion to the emergence of food products from GMAs, the EU supported for 3 years (2009–12) a project, Pegasus, (<http://www.projectpegasus.eu>) to provide consumers with a complete analysis of several projects in course. The Pegasus project included two-citizen

juries to analyze more particularly two of the projects in course: the preparation of human polyclonal antibodies and the salmons with accelerated growth. The first project received the support of a majority of the participating citizens, not the second project. The motivation not to accept GMAs is obviously complex. GMOs appear as a symbol of a progress which might be not needed. This seems to reflect more the doubt of the developed society than GMO problem proper.

In the United States, the different groups of the society involved in the use of GMAs have taken official engagements to which they can refer in case of conflict ([Biotechnology Industry Organization, 2009](#)). EFSA and FDA have published guidelines aiming at protecting consumers and environment ([EFSA 2010a, b, 2011a, b; FDA, 2009](#)).

PERSPECTIVES

The technical progress to generate transgenic animals during the last decade is very significant. This is namely the case for the use of transposons, BAC, siRNA, and engineered nucleases allowing genome editing. The time when transgenic mice were prepared essentially in a blind manner by microinjecting simple gene constructions is over. The generation of GMAs including farm animals has become easier, cheaper, more precise, and safer ([Editorial, 2016](#)). It may thus be considered that the generation of GMAs is no more a bottleneck for most of the biotechnological applications.

The number of transgenic models for basic research is going to be extended accordingly to a larger number of species, namely to rats, ruminants, chicken, and xenopus. More animals will be generated and thus sacrificed but the amount of relevant basic and medical information per animal should increase. The number of animals required for the preparation of pig organs to be transplanted to humans and the production of pharmaceutical proteins will remain whatever happens very limited. These animals will be bred in very specialized facilities not connected to farms.

The salmons with accelerated growth are on the market, opening an avenue for aquaculture. The other projects as the production of rhLf and rhLys in milk are making slow progress. This is the case also for most of the other projects in course. The chicken resistant to influenza will likely be on the market. Indeed, this project is relevant for animal and human health with no particular risk. The dissemination of these animals in farms should also be facilitated by the fact that chicken reproduce rapidly and that the market of these animals is highly organized.

It seems therefore that the public opinion will accept GMAs for food production if they see a clear advantage for them, for the animals, and for environment (http://cordis.europa.eu/result/report/rcn/58569_en.html). The generation of animals resistant to diseases appears globally beneficial in most cases. According to some experts, the trends for the production of GM

farm animals could be based on the use of the following genes: genes coding for digestive enzymes, genes stimulating feed ingestion, genes reducing fat content in milk and meat, genes reducing lactose content in milk, genes reducing CH₄ and CO₂ release, and genes reducing heat production. The impact of GMAs on human activities and environment seems not unacceptable (Houdebine, 2014).

Transgenic drosophila started being used more than two decades ago, essentially for basic research. Transgenic silkworms have been obtained more recently, mainly for the possible preparation of recombinant proteins and to obtain lines resistant to some diseases. Studies in course aim at producing artificial silk having different applications. Important projects aiming at inhibiting insects which are carriers for various pathogens are in course. Most of them rely on the massive generation and the voluntary dissemination of sterile genitors. These sterile males prevent the fertilization of females. Their sterility offers also the great advantage of making impossible their dissemination in environment. The first trials in fields started recently, and the preliminary results strongly suggest that this strategy could represent a major means to eradicate some diseases (Corby-Harris et al., 2010; Deredec, Godfray, & Burt, 2011; Kokoza et al., 2010; Scolari et al., 2011; Simmons et al., 2011; Subbaraman, 2011; Tabashnik et al., 2010; Gantz et al., 2015).

GM pets could represent an important market in the future. The GloFish story is an example. These animals, zebra fish, show various colors under UV light. Such animals have been obtained independently by different companies. They have been put on the market without any agreement of FDA which considered that these fish was neither food nor feed. The GloFish are tropical animals which could survive in environment. Some pet owners wish to have animals no more expressing genes coding for allergens or giving resistance to some diseases. It is clear that most of these animals would disseminate their transgenes without any control.

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Chapter 6

Microbial Biotechnology and Sustainable Agriculture

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INTRODUCTION

Biotechnology is the rapidly growing segment in biological sciences and has diversified applications in sustainable agriculture. This deals with the manipulation through genetic engineering (GE) of living organisms or their components to produce useful products for different applications in biological sciences. At the end of 2033, the increased world's population creates demand for food and shelter. This poses a great challenge to agricultural system to solve the problem of food demand (Mostafiz, Rahman, & Rahman,

2012). According to Barea (2015), demand for agricultural production is expected to increase by at least 70% by 2050. At that time, people become aware about the agricultural demands, food insecurity, and at the same time, they will come to know that sustainable agricultural practices are fundamental for future agricultural demand (Barea, 2015). Microbial biotechnology is an important area that promotes advances in food safety, food security, value-added products, human nutrition, functional foods, plant and animal protection, and overall fundamental research in the agricultural sciences.

The genetic resources of plants, animals, and microbes constitute the raw material for all biotechnology-based research, technology development, innovation improvement, and creation of new products. All the processes of genetic resources, namely collection, conservation, evaluation, and utilization, have been eminently impacted by biotechnology. The molecular tools of biotechnology have accelerated precision breeding by identifying, isolating, cloning, and transfer of desired genes from one species to another, rendering the concept of Mendelian population as an obsolete concept. The detecting single-nucleotide polymorphism, identifying functions of specific genes, assigning functions to unknown genes, and developing improved transgenic with specific fruitful desired character are the ultimate goals of biotechnology (Singh, 2000).

Agricultural practices are as of now implemented on a worldwide scale and diverse methodologies are being routed to meet sustainable environmental and economical developments, with the final aims of maintaining yield while safeguarding the biosphere. Therefore, the crossing point of economy–environment (agroecology), environment–society (environmental awareness), and society–economy (life standard) finally determines the concept/action of “sustainable development.” A target in sustainability is to find out efficient methods for recycling nutrients, controlling pest and pathogens, and for lightening the negative impact of abiotic stress variables, essential issues for human life, and for the sustainability of global ecosystems. So, the role and management of the root-associated microbiome is essential to meet both economical and ecological sustainable issues (Barea, 2015).

Agricultural microbiology is introduced as a synthetic research field responsible for the exchange of knowledge from general microbiology and microbial ecology to the agricultural biotechnology. Analysis of the regular circulation of microorganisms between plants, animal, and soil-borne niches are required to reconstruct the arrangement of the microbiota in natural and agricultural ecosystems.

A transgenic plant is one that contains a gene or genes which have been introduced artificially into the plant's genetic makeup using a set of several biotechnology techniques collectively known as recombinant DNA technology. DNA spliced to the coding portion of the genes that serves to regulate how they function is also transferred into the host plant. The inserted genes are known as transgene when they are inserted into the new host plant. The

transgene may come from another plant of the same or a different species, or a completely unrelated kind of organisms like bacteria or animals. Some of the examples of genetically manipulated crops for global benefit are as follows: control ripening improves shelf life and quality (e.g., tomatoes, peas, peppers, tropical fruits, broccoli, raspberries, and melons), insect resistance to reduce insecticide use (e.g., tomatoes, potatoes, corn, rice, lettuce, coffee, cabbage family, and apples), fungal resistance to reduce fungicide use (e.g., peppers, tomatoes, and cucumbers), viral resistance reduces diseases caused by plant viruses and, since insects carry viruses, reduces use of insecticides (e.g., potatoes, tomatoes, cantaloupe, squash, cucumbers, corn, oilseed rape canola, soybeans, and grapes), and herbicide tolerance improves weed control (e.g., soybeans, tomatoes, corn, cotton, oilseed rape canola, and wheat).

Microbial biotechnology contributes to sustainable agriculture by reducing the dependence on agrochemicals, particularly pesticides, through the deployment of genes conferring tolerance or resistance to biotic and abiotic stresses. Carefully selected genes from related or unrelated genetic resources are integrated in otherwise desirable genotypes. Biotechnology has been contributing to sustainable agriculture through the following ways:

1. Increased resistance against biotic stresses (insect pests and diseases);
2. Increased resistance against abiotic stresses (drought, cold, flooding, and problem soils);
3. Bioremediation of polluted soils and biodetectors for monitoring pollution;
4. Increased productivity and quality;
5. Enhanced nitrogen fixation and increased nutrient uptake and use efficiency;
6. Improved fermentation technology;
7. Improved technologies for generating biomass-derived energy;
8. Generation of high nutrient levels in nutrient-deficient staple crops such as rice.

It is a popular opinion that the most promising strategy to reach this goal is to substitute hazardous use of mineral fertilizers, pesticides in agriculture with environment-friendly symbiotic microbes, which could improve the nutrition of crops, as well as their protection against biotic (pathogens and pests) and abiotic (including pollution and climatic change) challenges.

Agriculture encompasses the entirety of the system that grows, and provides food, feed, fiber, ornamentals, and biofuel for the nation. Agriculture incorporates the management of natural resources, for example, surface water and groundwater, forests and other lands for commercial or recreational uses, and wildlife; the social, physical, and biological environments; and the public policy issues that relate to the overall system. All activities, practices, and procedures of general society and private sectors are involved in agriculture and forestry.

Not only do cultivating ventures reflect many combinations of farming practices, association structures, and management strategies, but also a wide

range of frameworks can conceivably add to accomplishing different sustainability goals. Examples of practices designed to improve environmental execution of traditional agriculture are as follows:

Crop rotation

1. Cover crops
2. Reduced-tillage and no-till practices
3. Integrated pest management
4. Precision farming
5. Diversification of farm enterprises
6. Other agricultural conservation best management practices
7. The development of crops and animals that have improved genetic resistance to climatic extremes, pests, and other threats, frequently with the utilization of new GE tools.

Some farming approaches have been developed, at least in part, to respond to perceived problems associated with conventional farming. Such approaches emphasize the utilization of natural processes within the farming system, often called “ecological” or “ecosystem” strategies, which fabricate productivity (and ideally resilience) through complementarities and cooperative energies inside the field, the farm, and at bigger scales across the landscape and community. Examples of ecologically based farming systems are as follows:

1. Organic farming systems
2. Biodynamic farming systems

In its broadest sense, sustainability has been described as the ability to provide for core societal needs in a manner that can be readily continued into the indefinite future without unwanted negative effects. The sustainability of a farming practice or system could be evaluated on the basis of how well it meets various societal goals or objectives.

1. Satisfy human food, feed, and fiber needs, and contribute to biofuel needs.
2. Enhance environmental quality and the resource base.
3. Sustain the economic viability of agriculture.

Enhance the quality of life for farmers, farm workers, and society as a whole.

THE MICROBIOME

Potential Significance of Beneficial Microbes in Sustainable Agriculture

A major focus in the coming decades would be on safe and ecofriendly methods by exploiting the beneficial microorganisms in sustainable crop production. The soil matrix is the major reservoir of microorganisms that interface

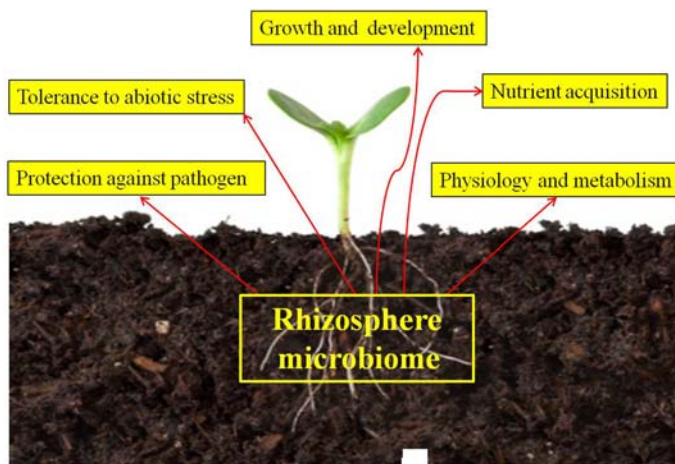


FIGURE 6.1 Role of rhizosphere microbiome for sustainable agriculture.

with plants, being depicted as the most biodiverse ecosystem on Earth. The soil microbiome is responsible for imperative processes occurring in this environment, which specifically relate to plant well-being. Several functions have been credited to microbiome in close relationship with plants, for example, their capacity to provide nutrients (phosphorus solubilization and nitrogen fixation); their support of nutrient uptake from the soil; and their ability to promote plant protection (Fig. 6.1). Organic farming is most dependent on the natural microflora of the soil which constitutes all kinds of useful bacteria and fungi including the arbuscular mycorrhiza fungi called plant-growth-promoting rhizobacteria (PGPR). A key advantage of beneficial microorganisms is to assimilate phosphorus for their own requirement, which in turn is available as its soluble form in sufficient quantities in soil. The microorganisms *Bacillus*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, *Fusarium*, *Sclerotium*, *Aspergillus*, and *Penicillium* have been reported which are active in the solubilization process. Abiotic and biotic stresses are the major constraints that are affecting the productivity of the crops. Many tools of modern science have been broadly connected for crop improvement under stress, of which PGPRs role as bioprotectants has turned out to be central significance in such manner (Yang, Kloepper, & Ryu, 2009).

Plant–Microbe Interactions

Microorganisms can affect agricultural productivity, for instance, by assisting and controlling nutrient availability/acquisition and promoting stress tolerance. The richness of species and diversity of microbial communities that comprise the plant microbiome, as well as the factors affecting it and their performance, are mostly unknown. The importance of this subject is

evidenced by the growing number of scientific publications on this topic in recent years, as well as research focusing on specific niches of plants and how they modulate their specific microbial communities. It is important for a better understanding of the most important drivers of the composition of plant microbiome, which is an active component of the host, also responsive to changes in environmental (biotic and abiotic) conditions. In order to understand the factors that influence this assembly and the dynamics from a phylogenetic and functional perspective, recent studies have focused on various parts of the plant microbiome separately. Partitioning the plant microbiome considers three noteworthy compartments where microbial cells can establish and develop: the so-called rhizosphere, endosphere, and phyllosphere (Hirsch, Miller, & Dennis, 2013). In spite of the fact that the plant microbiome is perceived as a tremendous fortune trove of microbial diversity, various important crop species and their natural relatives have not yet been studied for their associated bacterial communities. To better understand the significance of the plant-associated microbiome in the prevention of pathogen, outbreaks have been reported (Andreote, Rocha, Araújo, Azevedo, & Overbeek, 2010).

The lack of appropriate methodology has constrained advances for a comprehensive understanding of the mechanisms underlying plant–microbe interactions in the rhizosphere. Challenges depend primarily on the need of profiling an awesome cluster of procedures where the vast and different microbial communities are predominantly constituted by uncultivable microorganisms (Carvalhais, Dennis, Tyson, & Schenk, 2013). These techniques, based on molecular approaches, are likewise major to assess the effects of bothers incited by biotic and abiotic stress factors on soil microbiome diversity and on plant–microbe interactions, in the present situation of global change. Improving the ability of soil microbes for stress alleviation in crops is based on a better understanding of plant–microbiome interactions (Barea, 2015). Diverse types of stress factors, including salinity, drought, nutrient deficits, contamination, diseases and pests, etc., can alter plant–microbe interactions in the rhizosphere.

Scientist discovered that the presence of even small amount of water can influence the structure of plant roots in soil, a finding that opens up new possibilities to improve water and nutrient aging for important food crops. The degree of root branching determines the efficiency of water uptake and acquisition of nutrients in crops. Understanding the regulation of root branching is therefore of vital importance.

Microbial Interactions Across Plant, Soil, and Environmental Interfaces

Plants are associated with a continuum of other organisms, the majority of which are microbes. These include epiphytes on plant surfaces, endophytes within plants, rhizosphere, and soil microbes associated with subsurface plant

organs and soil interfaces. Symbiosis between legume plants and *Rhizobia* in the soil is of specific significance in agriculture, and more research has been centered on characterization of the molecular mechanisms that establishes species-specific collaboration (Pinto et al., 2014). Legume—*Rhizobia* interactions are mediated by host-specific flavonoids secreted in the root exudates. Numerous rhizosphere microorganisms can incite a systemic response in plants, activating plant defense mechanisms. Inoculation with nonpathogenic root zone bacteria can trigger signaling pathways that prompt to higher pathogen resistance of the host, the so-called induced systemic resistance (ISR). Several bacteria that have been used to study beneficial effects under abiotic stress conditions, such as *Bacillus* sp., have been shown to induce ISR. Bacterial endophytes, used for biological control of various plant diseases and for improved plant agronomic characteristics, might be of particular interest as they have the advantage of being relatively protected from the competitive soil environment; besides, they usually grow in the same plant tissue where bacterial plant pathogens are identified.

TYPES OF ROOT-ASSOCIATED MICROORGANISMS

Plant-Associated Microbiome

The plant-associated prokaryotic bacteria and the eukaryotic fungi have an awesome assortment of trophic/living habits whose saprophytic or symbiotic associations with the plant could be either detrimental or beneficial. The vast majority of these microorganisms remain in the rhizospheric soil or rhizoplane, yet a little subpopulation of them, assigned as “endophytes,” is able to penetrate and live within plant tissues (Mercado-Blanco, 2015).

Soil microbes are recognized as a relevant component within the diverse interacting factors responsible for the environmental quality required for a sustainable healthy food production. Microbes are attracted to, and maintained at, rhizosphere microhabitats by the rhizodeposit pools (Hirsch et al., 2013). The soil microbiome contains different sorts of organisms, but bacteria, fungi, and archaea are those receiving by far more consideration in soil microbiology studies.

Beneficial Rhizosphere Microorganisms

Beneficial saprophytic rhizosphere microbes enhance plant performance acting as: (1) decomposer of organic substances (detritus); (2) PGPR; or (3) antagonists of plant pathogens. The PGPR are known to participate in numerous vital ecosystem processes, such as the biological control of plant pathogens and nutrient cycling. Beneficial plant mutualistic symbionts include the N_2 -fixing bacteria and the multifunctional arbuscular mycorrhizal (AM) fungi. Bacteria belonging to diverse genera, collectively termed

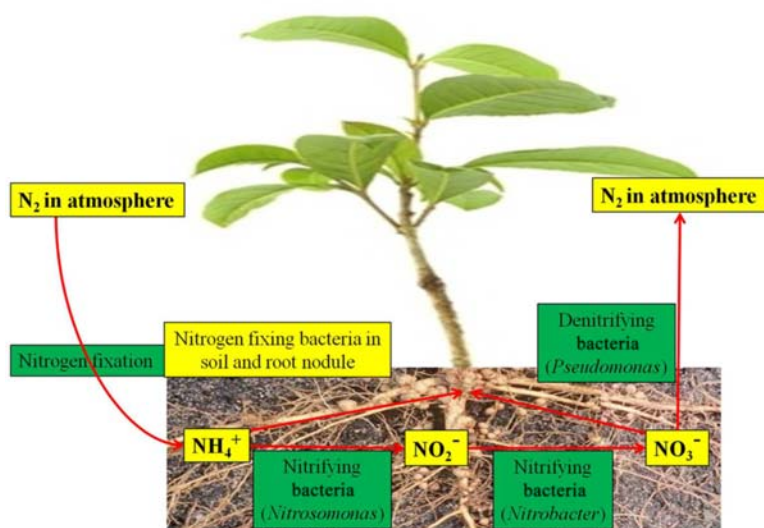


FIGURE 6.2 Nitrogen fixation by rhizospheric microorganism.

“rhizobia,” are able to fix N_2 in mutualistic symbiosis with legume plants. The atmospheric nitrogen is converted into plant-utilizable forms (ammonia and nitrate) by nitrogen-fixing microbes which changes nitrogen to ammonia (Fig. 6.2). Agriculturally important microfloras are often designated as a large group of frequently unknown or ill-defined microorganisms that interact favorably in soils and with plants to provide beneficial effects which are sometimes difficult to predict.

Plant-Growth-Promoting Rhizobacteria

The plant–microbe interactions in the rhizosphere assumes a critical part in transformation, mobilization, solubilization, and so forth of nutrients from a restricted nutrient pool, and along these lines uptake of essential nutrients by plants to understand their full genetic potential. The utilization of PGPR has found a potential role in developing sustainable frameworks in crop production. The PGPR are characterized by the some inherent uniqueness such as they must be proficient to colonize the surface of the root, or superficial intercellular spaces of the host plant; they must promote plant growth; they must survive, multiply, and compete with native microbiota in rhizosphere microhabitats at least for the time needed to express their beneficial plant-growth promotion activities. PGPR additionally help in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, and improve soil structure and organic matter content. PGPR hold more soil organic

nitrogen, and other nutrients in the plant–soil system, accordingly they help in decreasing the requirement for nitrogen and phosphate fertilizer and enhance release of the nutrients.

Role played by PGPR

1. Preservation of natural resources and environment
2. Phosphorous solubilization
3. Production of plant-growth regulators
4. Potash mobilization
5. Microorganisms as biofertilizer and biopesticide
6. Production of volatile organic compounds (VOCs)
7. Microbes as biotic elicitors
8. Microbial responses in stress agriculture
9. Microbial antagonism

Mechanism of Action of PGPR in Rhizosphere Region

PGPR-mediated-plant-growth promotion occurs by the alteration of the whole microbial community in rhizosphere niche through the production of various substances. PGPR promote plant growth directly by either encouraging resource procurement (nitrogen, phosphorus, and essential minerals) or balancing plant hormone levels, or indirectly by diminishing the inhibitory effects of different pathogens on plant growth and development in the forms of biocontrol agents (Fig. 6.3). The general mechanisms of plant nutrient management by microorganisms include associative nitrogen fixation,

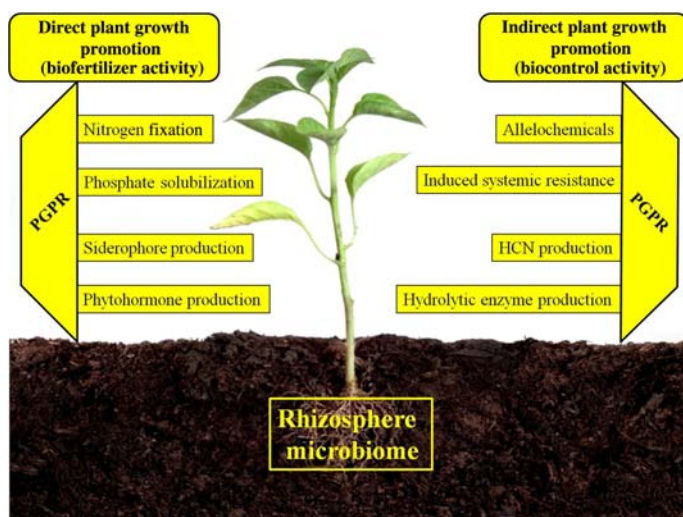


FIGURE 6.3 Mechanism of action of PGPR.

lowering of ethylene levels, production of siderophores, production of growth regulators, VOCs, solubilization of nutrients, and promotion of mycorrhizal functioning. Key processes in plant–bacteria interactions and colonization by inoculated strains still need to be unraveled to allow full-scale application of bacteria-assisted phytoremediation of trace-element-contaminated soils.

Application of High-Quality Microbial Inoculants

Recently, [Bashan, de-Bashan, Prabhu, and Hernandez \(2014\)](#) have published a comprehensive review on the formulation and practical perspectives of inoculants technology for PGPR. They recommend a number of top priorities of research to implement delivery systems for PGPR and rhizobia. For the successful application of microbial inoculants in agriculture, the following aspects need to be implemented: (1) to increase the scientific/technological bases of inoculums production and application; (2) to generate specific normative for each inoculants type and its application, either on the seeds or on the soil, or to the plant to be transplanted already microbized; (3) to set up quality-control protocols; (4) to minimize the fluctuation of the field results; and (5) to expand information and dispersal by explaining advantages and limitations, and benefits for society.

Seed Treatments for Sustainable Agriculture

Seed is a basic and fundamental requirement for sustainable growth in agricultural productivity, as 90% of the food crops are propagated from seed. The seed-borne, early season diseases and insects produce devastating effect if not handled timely. Emphasis on present-day agriculture is to produce more with lesser land, water, and manpower. The age-old environment-friendly disease management practices like sanitation, crop rotation, mixed cropping, adjustment of date of sowing, fallowing, summer ploughing, green manuring composting, etc. to combat plant pathogens have already lost their acceptability and are being reevaluated as a component of integrated pest management.

Encourage Beneficial Microbe Establishment at Rhizosphere

Undoubtedly, getting biased rhizosphere opens new opportunities for future agricultural developments based on exploiting the beneficial microbial services to decrease the inputs of agrochemicals along these lines achieving sustainable environmental and economical goals.

1. Harnessing the rhizosphere microbial communities through agricultural managements.
2. Learning how plants shape microbial community structure in the rhizosphere.

3. The “biased rhizosphere” concept/action.

Several methodologies are at present routed to determine whether the rhizosphere can be engineered (biased) to empower beneficial organisms, while preventing presence of pathogens. The objective research themes offer many difficulties on the grounds that there are many crevices in our understanding.

Beneficial Microbes in Agriculture Under Changing-Climatic Scenario

Climate change is one of the major global problems, affecting the life on the planet Earth. Climate change usually affects the photosynthesis (carbon assimilation), root activity, general morphology, and functioning of the plant specimens as well as their interactions. Increasing atmospheric carbon dioxide (CO₂) reduces crop nitrogen content, which may retard many pests and diseases and thereby causes change in the composition of weed flora that accompanies the crops. Due to excessive and injudicious use of chemical fertilizers, agriculture faces diverse challenges (Bhattacharyya, Sarmah, Dutta, & Tanti, 2015). Synthetic fertilizers are known to wipe out a large percentage of soil's naturally occurring essential micro- and macronutrients. Change in environmental conditions due to climate alterations is likely to induce changes in plant physiology and root exudation. The research is essential to improve the knowledge on native biodiversity and microbial community structure, under changing-climate scenario.

BIOFERTILIZERS

Biofertilizers are the microbial inoculants which can be usually defined as a preparation containing live or dormant cells of efficient strains of nitrogen fixing, phosphate solubilizing, and cellulytic microorganisms, etc. In contrast to chemical fertilizers, biofertilizers are viable microorganisms which are not the source of nutrients but provide help to plants in accessing the nutrient availability in rhizospheric region. Several microorganisms are commonly used as biofertilizers including nitrogen-fixing soil bacteria (*Azotobacter*, *Rhizobium*), nitrogen-fixing cyanobacteria (*Anabaena*), phosphate-solubilizing bacteria (*Pseudomonas* sp.), and AM fungi. Similarly, phytohormone (auxin)-producing bacteria and cellulolytic microorganisms are also used as biofertilizer formulation. These microbial formulations are used to enhance certain microbial process to increase the availability of nutrients in a form which can be assimilated by plant. Biofertilizers are low-cost, renewable sources of plant nutrients. These are the strains of beneficial soil microorganisms which are cultured and packed in suitable carrier in laboratory. A carrier is a material, such as peat, lignite powder, vermiculite, clay, talc, rice bran, seed, charcoal, soil, rock phosphate pellet, paddy straw compost, wheat

bran, or a mixture of such materials, etc. which provides better shelf life to biofertilizer formulation.

Recently, biofertilizers are gaining momentum due to the vast advantages such as maintenance of soil health and reduction of environmental pollution by using of the chemicals in agriculture (Muraleedharan, Seshadri, & Perumal, 2010). Increased crop yield largely depends on the type of fertilizers used to increase essential nutrients for plant growth and development. For optimal plant growth, it requires nutrients in sufficient and balanced quantities but from the soil only a small portion of nutrients are released every year through biological or chemical processes. Therefore, aim of fertilizers use is to supplement the nutrients already exist in the soil. Besides nutrient supplementation, biofertilizers have various other benefits for example control soil-borne diseases, improves soil health, soil properties and result in higher yield rates. At present, a variety of commercial biofertilizer formulations are available, and diverse strategies have been adopted to make sure maximum viability of the microorganisms used in such formulations. The strategies comprise (1) optimization of biofertilizer formulation, (2) application of liquid biofertilizer, and (3) application of thermotolerant/drought-tolerant/genetically modified strains. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function (Table 6.1).

Mycorrhiza is a well-known association of fungus with the roots of higher plants. Although it remains mystery, it serves as a model system to understand the mechanism behind stimulation of growth in the root cells as a result of mycorrhizal inhabitation. Bioactive ligands called Myc factors and Nod factors secreted by mycorrhiza and rhizobium were perceived by host roots to trigger the signal transduction pathway, which initiates further signal transduction pathway through unknown receptors (SYMRK and NORK) which trigger release of Ca^{2+} in the cytosol. The whole pathway involves receptor like kinases or other kinase-related proteins like DMI and SYM71 to phosphorylate their substrates. Nuclear pore complex and some of its proteins (NUP) play a role in calcium spiking. DMI proteins play a role in maintaining periodic oscillation of calcium ions inside and outside the nucleus. Several channel proteins (Ca^{2+} channel proteins) also facilitate this process with the help of various transporters. CcAMK is a calcium calmodulin-dependent protein kinase, which phosphorylate the product of CYCLOPS protein thus initiating activation of various genes involving formation of structures like nodule and prepenetration apparatus.

BIOPESTICIDES

The biopesticides are second hand as microbial biological pest oversee agents which are applied in farming processes to replace chemical pesticides

TABLE 6.1 Grouping of Biofertilizers With Functions

S. No.	Nature of Organisms	Functions	Examples
1.	Free-living	N ₂ -fixing biofertilizers	<i>Azotobacter</i> , <i>Beijerinckia</i> , <i>Clostridium</i> , <i>Klebsiella</i> , <i>Anabaena</i> , and <i>Nostoc</i>
2.	Symbiotic		<i>Rhizobium</i> , <i>Frankia</i> , and <i>Anabaena azollae</i>
3.	Associate symbiotic		<i>Azospirillum</i>
4.	Bacteria	P-solubilizing biofertilizers	<i>Bacillus megaterium</i> var <i>phosphaticum</i> , <i>Bacillus subtilis</i> , <i>Bacillus circulans</i> , and <i>Pseudomonas striata</i>
5.	Fungi		<i>Penicillium</i> sp. and <i>Aspergillus awamori</i>
6.	Arbuscular mycorrhiza	P-mobilizing biofertilizers	<i>Glomus</i> sp., <i>Gigaspora</i> sp., <i>Acaulospora</i> sp., <i>Scutellospora</i> sp., and <i>Sclerocystis</i> sp.
7.	Ectomycorrhiza		<i>Laccaria</i> sp., <i>Pisolithus</i> sp., and <i>Boletus</i> sp.
8.	Ericoid mycorrhizae		<i>Pezizella ericae</i>
9.	Orchid mycorrhiza		<i>Rhizoctonia solani</i>
10.	Silicate and zinc solubilizers	Biofertilizers for micronutrients	<i>Bacillus</i> sp.
11.	<i>Pseudomonas</i>	Plant-growth-promoting rhizobacteria	<i>Pseudomonas fluorescence</i>

(Table 6.2). Biotechnology could as well condone in developing alternative controls to synthetic insecticides to fight against insect pests. Formulas for coatings these beneficial organisms could be developed to protect the plant during the critical seedling stage. Continually, second-hand biopesticides are bacterial and fungal agents such as *Trichoderma* spp., *Ampelomyces quisqualis* (a control agent for grape powdery mildew), and *Bacillus subtilis* (used to control plant pathogens). Agile property reckons a microorganism that acts as a biocontrol agent, directly affecting the pathogen (e.g., Contans), or produces a compound during fermentation that provides control (e.g., Sonata).

TABLE 6.2 Categories of Microbial Biopesticides

Category	Microorganisms	Target Pest	Mode of Action
Bactericide	<i>Agrobacterium radiobacter</i>	Crown gall (<i>Agrobacterium tumefaciens</i>)	Antagonist and antibiosis
	<i>Bacillus polymyxa</i>	Crown gall	Antagonist and antibiosis
	<i>Bacillus sphaericus</i>	Crown gall	Antagonist and antibiosis
	<i>Bacillus subtilis</i>	Bacterial pathogen	Colonizes on plant root and competes
	<i>Pseudomonas fluorescens</i>	Several bacterial diseases such as frost-forming bacteria	Crowds out and controls the growth of plant pathogens
Fungicide	<i>Bacillus subtilis</i>	Soil foliage, fungal pathogens such as <i>Rhizoctonia</i> , <i>Fusarium</i> , <i>Aspergillus</i> , and others	Colonizes on plant root and competes and antibiosis
	<i>Pseudomonas syringae</i>	Postharvest disease	Utilize seed exudates, produce a wide spectrum of bioactive metabolites
	<i>Bacillus pumilus</i>	Seedling disease	Colonizes on plant root and competes and antibiosis
	<i>Streptomyces</i>	Fungi-causing damping off, stem, and crown rots	Mycoparasitic, antagonist, and antibiosis
	<i>Pseudomonas fluorescens</i>	Plant soil-borne diseases, fireblight	Utilize seed and root exudates and colonize, produce a wide spectrum of bioactive metabolites
	<i>Trichoderma viride/harzianum</i>	Soil-borne fungal disease	Mycoparasitic, antagonist, and antibiosis
	<i>Burkholderia cepacia</i>	Fungal pathogens	Controls fungi via seed treatment
	<i>Gliocladium catenulatum</i>	Seed-borne and soil-borne diseases	Enzymatic mechanism
	<i>Candida oleophila</i>	Postharvest pathogens	Colonization of diseased tissues
(Continued)			

TABLE 6.2 (Continued)

Category	Microorganisms	Target Pest	Mode of Action
Insecticide	<i>Bacillus thuringiensis</i> (Bt)	Butterfly & moths lepidoptera	Digestive system
	<i>Beauveria bassiana</i>	Foliar-feeding insects	White muscadine disease
	<i>Paecilomyces fumosoroseus</i>	Whitefly and thrips	Parasitic
	<i>Metarhizium anisopliae</i>	Coleoptera and lepidoptera, termites, mosquitoes, leafhoppers, beetles, and grubs	Penetrate the exoskeleton of the insect and grow directly through the cuticle to the inner body of their host
	<i>Verticillium lecanii</i>	Whitefly, coffee green bug, and homopteran pests	Grow directly through the cuticle to the inner body of their host
	<i>Lecanicillium longisporum</i>	Aphids	Grow and invade the body of insects
	<i>Nucleopolyhedrosis virus</i> (NPV)	Species of Lepidoptera, Hymenoptera, and Diptera	Infect digestive cells in larvae gut
	<i>Granulosis virus</i> (GV)	Species of Lepidoptera	Infect digestive cells in larvae gut
Herbicide	<i>Xanthomas campestris</i>	Annual bluegrass	
	<i>Alternaria destruens</i>	Herbicide—dodder	
	<i>Colletotrichum gloeosporioides</i>	Herbicide—northern Jointvetch	
	<i>Chondrostereum purpureum</i>	Herbicide—stump sprout inhibitor	
	<i>Phytophthora palmivora</i>	Strangler vine	
	<i>Puccinia thlaspeos</i> woad	Herbicide—Dyer's woad	
Nematicide	<i>Bacillus firmus</i>	Nematodes	Competes, antibiosis
	<i>Paecilomyces lilacinus</i>	Nematodes	Infect and destroy nematode's eggs
	<i>Myrothecium verrucaria</i>	Nematodes	Paralyzing the muscle of nematodes that control the feeding and locomotion
	<i>Verticillium chlamydosporium</i>	Nematodes	Infect and destroy nematode's eggs

The justification for invariable house of take meals has led conventional agriculture to be strongly dependent on chemicals. The extend liaison of clientele and charge on go aboard look after has led growers to explore new environmentally friendly methods to replace, or at least supplement, the current chemical-based practices. The interest of biopesticides has emerged as a promising alternative to chemical pesticides. The efficacy of biopesticide bacteria such as *Bacillus circulans*, *Agrobacterium radiobacter*, *Bacillus pumilus*, and *Pseudomonas aureofaciens* and fungi such as *Ampelomyces quisqualis*, *Fusarium oxysporum*, *Gliocladium virens*, *Trichoderma harzianum*, and *Pythium oligandrum* was utilized by many countries for their growth in the field of agriculture for sustainable development (Hynes & Boyetchko, 2006).

ROLE OF BIOTECHNOLOGY FOR A SUSTAINABLE AND SAFE GLOBAL AGRICULTURE

Biotechnology offers great opportunities to increase global agricultural production and strategies to protect our environment through reduced use of agrochemicals like pesticides, fertilizers, rodenticides, etc. Biotechnology has played a pivotal role toward the achievement of environmental sustainability by using ecofriendly crops such as herbicide tolerant, insect-resistant species and crops that can fix atmospheric nitrogen leading to purification of the environment. The significance of these novel technologies such as biotechnology in food security, environmental sustainability, and economic development was accepted at the United Nations General Assembly in 2005 (Ene-Obong, 2007).

Some of the advanced microbial biotechnological applications applied in large scale are as follows:

1. Removal of toxic chemicals from the environment,
2. Removal of heavy metal pollution from the environment,
3. Desulphurization of fossil fuels,
4. Ecosystem modeling,
5. Monitoring of environmental pollution,
6. Bioassay of environmental toxicity,
7. Control of oil spillage, and
8. Saving of resources and energy.

Although food security in a global context is complex issue and depends on many infrastructural, socioeconomic, and political factors. The amazing ability of agricultural sector to maximize crop yield and production without compromising the bioresource assets will also be important. Agricultural biotechnology offers great potential for promoting sustainable agriculture (Anderson et al., 2016). Traits developed to help improve yield, reduce inputs, and provide protection from viral, bacterial, fungal pathogens, and

insect pests, as well as to enhance crop performance and productivity under biotic and abiotic stress conditions, are as follows:

1. Genetically modified virus-resistant common beans
2. Semiochemicals as new targets for GM crops
3. Engineering abiotic stress tolerance in crop plants
 - a. Nitrogen-use efficiency
 - b. Salt-tolerance technology
 - c. Drought-tolerance and water-use efficiency technology
 - d. Combined technologies
4. Genetically modified (GM) bananas resistant to *xanthomonas* wilt (bxw) disease
5. Virus-resistant cassava for Africa
6. Using life cycle assessment to assess environmental and socioeconomic impacts of GM crops.

Probiotics

Probiotics are selected viable microbial dietary supplements that, when introduced in adequate quantities, beneficially affect human being through their effects in the intestinal tract (Holzapfel & Schillinger, 2002). The term "probiotic" means "for life," and it was described by an expert committee as "live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition" (FAO/WHO, 2001). Probiotics have been used since several years in fermented dairy products. However, the possible uses of probiotics in nondairy food products and agriculture have not received formal attention. A blend of probiotic bacteria with nutrient-dense foods, for example dairy products, will have the positive benefit in enhancing consumer nutrition. In present scenario, there has been an increased interest to food and agricultural applications of probiotics, the selection of new probiotic strains, and the development of new application has gained much importance. Probiotics have become a very important nutritional factor to daily health food products, and their worldwide market is estimated above US\$28.8 billion by 2015. The agricultural applications of probiotics in animal, fish, and plants production have increased day by day. The nonpathogenic organisms used as probiotics consist of a wide variety of microbial strains and have ability to adhere, colonize, and modulate the human gastrointestinal tract. *Lactobacillus* and *Bifidobacterium* are the major probiotic groups but there are some microbial strains such as *Pediococcus*, *Lactococcus*, *Bacillus*, and yeasts. which are also reported with probiotic potential. Some of the identified probiotic strains exhibit powerful antiallergic, antiinflammatory, and other important properties.

GENETICALLY ENGINEERED CROPS: CONTRIBUTION TO SUSTAINABLE AGRICULTURAL SYSTEMS

World is facing serious challenges in the areas of food, health, environment, and energy. To address these challenges, plant genomic is one of the key areas to overcome all these fundamental needs through improving plant genetics and plant–environmental interactions to play essential roles in meeting the chronic demands of global food security.

Genetically Engineered Crops

GE is also called genetic modifications, which deliberates certain characteristics of an organism by manipulating the genetic material of particular plant. GE is also differing from conventional methods of genetic modification in two interesting ways: (1) introduce one or a few well-characterized genes into plant and (2) introduce genes from any species into a plant. Number of crops developed on the basis of genetic manipulation strategies to overcome the food loss and provide food with sufficient nutrition to world. The examples are as follows:

1. Insect-resistant crops [*Bacillus thuringiensis* (Bt cotton)]
2. Herbicide-tolerant crops
3. Viral-resistant crops

SUSTAINED AGRICULTURE THROUGH AGROECOSYSTEM

Biodiversity refers to all species of plants, animals, and microorganisms existing and interacting within the ecosystem. Natural biodiversity has provided the foundation for all agricultural plants and animals. Biological basis of agriculture can be sustained, but this will require both managing the agroecosystem and changing the biological players. In light of this, we focus on three key areas which include (1) managing plant rhizosphere processes, (2) the potential and limitations for modifying these processes, and (3) the contributions and limits of biotechnology in improving productivity from transgenic plants. Sustainable crop production will require build-up and maintenance of soil organic matter (SOM), formation of water-stable soil aggregates, increased microbial transformation of nutrients in the rhizosphere, selection of locally adapted crop and microbial ecotypes, and improved resistance of crops to pests and disease. Thus, two key areas of research include the ecological management of rhizosphere processes, especially the interactions between microorganisms and plant roots and enhanced genotype–environment matching with crops through the use of native genetic resources and cautious application of biotechnology. Long-time stability and

yield can be achieved through sustainable management by external inputs; they are as follows:

1. **Soil fertility:** It is one of the most important inputs for the particular crop. Soil represents a highly heterogeneous environment for the microbiota inhabiting it; the different figures of the solid fractions in soil (sand, silt, clay, and organic matter) provide myriads to different indigenous microbes. Microorganisms collectively are the underlying catalysts of the biochemical processes in soil. These processes are susceptible to major changes in the surroundings, whereby a measurable effect will be the result of individual shifts at the micrometer scale. SOM and clay particles hold large stores of plant nutrients. Crop roots and residues improve soil fertility by stimulating soil-microbial communities and improving soil aggregation. This improved soil physical environment aids water infiltration, water holding, aeration and, ultimately, root growth, and plant nutrient.
2. **Soil organic matter (SOM):** This is specially the more labile components, plays the key role in maintaining soil fertility and structure in many soils. SOM is chemically heterogeneous, variously consisting of sloughed-off root cells, dead roots, exudates from living roots, microbial and invertebrate biomass, fungal hyphae, mucilages and polysaccharides, among other components. The more labile components, especially within the rhizoplane (on or close to the surface of roots), serve as a ready source of energy-rich carbon compounds necessary to support high levels of microbial activity.
3. **Soil biology:** Microbial pool activity contributes to plant nutrition through decomposition, ammonification, nitrogen fixation, and solubilizing phosphorus. Together with mucilages and polysaccharides from roots and microbes and the network of fungal hyphae, SOM is essential to the formation of water-stable aggregates, the fundamental building blocks of soil structure.
4. **Rhizosphere management:** Plant roots live in close association with diverse microbial communities selectively recruited from soil via root-exuded carbon: detrimental biota recruited by crop plant rhizospheres cause significant annual global losses.

In the developing countries, particularly in India, the basic problem of production is rural poverty and hunger. The agroecology could be used to resolve the population hunger problem via focusing on a system that may change small yield survival sloping agriculture to a large yield, profitable, and highly sustainable agriculture. It is one of the most practiced ways to enhance crop productivity using agroecology in different agroecosystems, which allows benefits to the second crop through the first crop via changing the environmental conditions for second crop (Tripathi, 2015).

CONCLUSION

Biotechnology is the rapidly growing segment and has diversified applications in sustainable agriculture. The genetic resources of plants, animals, and microbes constitute the raw material for all biotechnology-based research, technology development, and creation of new products. Agricultural practices are currently executed on a large scale, and different approaches are being addressed to meet sustainable environmental and economical developments, with the final aims of maintaining yield, while preserving the biosphere. Agriculture includes the management of natural resources such as surface water and groundwater, forests and other lands for commercial or recreational uses and wildlife; the social, physical, and biological environments; and the public policy issues that relate to the overall system. Climate change is one of the major issues affecting the life on the planet Earth. Natural biodiversity has provided the foundation for all agricultural plants and animals. It is one of the most practiced ways to enhance crop productivity using agroecology in different agroecosystems, which allows benefits to the second crop through the first crop via changing the environmental conditions for second crop.

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Chapter 7

Impacts of Climate Change on Agriculture and Food Security

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INTRODUCTION

Climate change refers to any significant change in the signature patterns of typical weather conditions of an area, region, or the entire earth, either due to natural variability and/or as a result of human activity, projected over

comparable time periods, which may be observed over tens, hundreds, or perhaps millions of years (Stocker & Intergovernmental Panel on Climate Change, 2013). Changes in climatic conditions are expressed in terms of radiative forcing (In the earth-atmosphere system, radiative forcing is a measure to express the influence of a factor in transforming the incoming and outgoing energy balance. It is an index emanating the significance of the factor as a potential mechanism of climate change.), which is employed to compare the wide range of human and natural drivers known to affect cooling or warming influences on global climate (Stocker & Intergovernmental Panel on Climate Change, 2013).

Apart from a number of natural factors (ocean currents, continental drift, the earth's tilt, volcanoes, comets, meteorites, etc.) responsible for climate change, increase in anthropogenic activities such as pollution, urbanization, industrialization, agricultural activities, change in land use pattern, deforestation, etc. leads to increase in the atmospheric concentrations of water vapor, carbon dioxide, methane, and nitrous oxide, all greenhouse gases (GHGs) which further accelerates the rate of climate change (Gillett, Arora, Flato, Scinocca, & von Salzen, 2012; Stott et al., 2016).

On the one hand, greenhouse effect happens to be a natural process that configures the earth's climate, by creating relatively warm and hospitable environment close to the earth's surface, supporting humans and other life forms. However, on the other hand, an increased level of GHGs (carbon dioxide (CO_2), water vapor (H_2O), nitrous oxide (N_2O), methane (CH_4), sulfur hexafluoride (SF_6), hydrofluorocarbons (HFCs), and perfluorocarbons (PFCs), etc.) due to anthropogenic activities is gradually contributing to the overall increase in earth's temperature, thereby leading to global warming (Stocker & Intergovernmental Panel on Climate Change, 2013; Tripathi, Tripathi, Chauhan, Kumar, & Singh, 2016).

The average global surface temperature (14.6°C) has been found to increase by 0.74°C , since the late half of 19th century, and it is further expected to increase by $1.4\text{--}5.8^\circ\text{C}$ by the end of 2100 AD displaying significant regional variations (Stocker and on Climate Change, 2013; Tripathi et al., 2016). Concentration of atmospheric CO_2 has increased from 280 to 395 ppm; concentration of CH_4 has increased from 715 to 1882 ppb, whereas concentration of N_2O has gone up from 227 to 323 ppb between the year 1750 and 2012. These gases viz. CO_2 , CH_4 , and N_2O hold the Global Warming Potential of 1, 25, and 310, respectively (Stocker & Intergovernmental Panel on Climate Change, 2013).

Approximately, 70% of people in the developing countries reside in rural areas, where agriculture is the biggest supporter of livelihoods. Agriculture encompasses cultivation of all forms of plants, animals, and different life forms including products for food/feed, fiber, energy, etc. to sustain and enhance human life. Global agriculture is encountering significant pressure to meet the demands of growing population. Anthropogenic activities in the recent past have degraded soil and water resources which in turn place

extensive strains on food security for populations across globe (Mainuddin, Kirby, & Hoanh, 2011). These conditions are likely to get worse by climate change. It has been documented that a global warming of less than 2.5°C may not have any significant impact on overall food production; however, a warming beyond 2.5°C may reduce global food supplies leading to higher food prices.

The impact of climate change on agricultural crop yields and productivity is projected to vary considerably. According to some reports, in addition, shifting monsoons, heat stress, and drier soils may lead to reduction in yield by approximately one-third in the subtropics and tropics, where crops exhibit their maximum heat tolerance. It is expected that midcontinental areas (e.g., the US grain belt, some wide sections of Asian midlatitude region, African sub-Saharan sections and few parts of Australia) will experience hotter and drier conditions. At the same time, due to longer growing seasons and increased precipitation, many temperate regions may show increase in crop yields and productivity; reports reveal that the growing season has already lengthened in the North America, the United Kingdom, Europe, and Scandinavia (Tripathi et al., 2016).

Furthermore, agriculture and land use change are leading sources of GHG emissions, globally. Application of fertilizers, livestock rearing and concomitant land clearing, affects both, the atmospheric level of GHGs, as well as, the capacity for carbon storage and sequestration. Hence, although progressing climatic changes are influencing agricultural production, opportunities are also presented by the sector itself, for emissions reductions. Apart from agricultural production, climate change will also have a significant footprint on proximate advantages, as well as, trade flows. It is likely that there shall be pronounced divergence among regions in terms of agricultural output. Under bird's eye view, countries falling within the tropics and subtropical zones, with developing economies, shall be losing in terms of agricultural production, whereas countries in temperate zones, sharing developed economies, are surmised to gain. Most of the developing countries are highly dependent on developed economies for the production and exports of agricultural goods, and climate change is anticipated to cause significant losses in terms of growth as well as export opportunities (Wheeler & von Braun, 2013).

In the light of the aforesaid context, present chapter is an attempt to put forth detailed and multipronged impacts of climate change on agriculture and different dimensions of food security.

EFFECT OF CLIMATE CHANGE ON AGRICULTURE AND SOIL PROPERTIES

As soil is the rooting medium of plants in general, hence any adverse climatic impact may affect physicochemical and biological properties of soil, thereby influencing both agricultural productivity and yield, accompanied by a shift in climate and agriculture zones (Mihailović et al., 2016). In order to

study the differential impacts of climatic changes on agriculture, a vivid consideration of the following points becomes indispensable.

Shift in Climatic and Agriculture Zones

The current buildup of GHGs in the atmosphere is inducing global climate shifts, affecting growing and agroecological conditions (Son & Bae, 2015). As it is expected that average temperatures will comparatively increase more near the polar region compared to the equator, any shift in climate zones shall be more conspicuous close to higher latitudes. The shift expected in the midlatitude regions (45 to 60 latitude), for each degree rise in temperature, is about 200–300 km. The present latitudinal climate belts are individually optimal for particular crops; hence, above-mentioned shifts may have a substantial impact on both agricultural as well as livestock production. Longer growing seasons may be experienced for those crops, in which temperature is the limiting factor, e.g., for every 1°C rise in average annual temperature, the Canadian prairies may experience lengthening of the growing season by approximately 10 days (Stocker & Intergovernmental Panel on Climate Change, 2013).

Impact on Agriculture Soil

Effect on Soil Organic Matter and Soil Fertility

An increase in temperature is likely to affect the quality of soil through reduction in soil moisture and water-holding capacity, two vital properties perturbing nutrient availability to agricultural crops. Soil-moisture deficit not only bears direct impact on crop productivity but also reduces crop yields affecting the availability and transport of soil nutrients (Yigini & Panagos, 2016). With increasing aridity, crop yields on soils in the developing countries have been found to decrease exponentially.

Soil organic matter is another important soil component, as it is capable of improving soil quality by conditioning the soil structure, improving nutrient storage and turnover, water-holding capacity, oxygen-holding capacity, and soil stability. Soil organic matter acts both as a source and sink of carbon in the biosphere in the era of climate change. Organic matter is the prime habitat for innumerable variety of soil microflora and microfauna, which play a crucial role in determining productivity and health of soils. Soil organic matter is enormously amenable to changes in soil temperature, moisture, land use and management, and a significant decline in organic matter levels reported in many soils during the last few decades has further increased the susceptibility to soil erosion (Yigini & Panagos, 2016).

Drought increases susceptibility to nutrient losses around the rooting zone through erosion. As nutrients are transported to the roots by water, a deficit in soil-moisture content will impair its diffusion over short distances

as well as the mass flow of water-soluble nutrients (e.g., sulfate, nitrate, Mg, Ca, Si) along longer distances. Roots alter their architecture by extending their length, increase in surface area in an effort to capture nutrients such as phosphorus which are less mobile. Under drought conditions, there is a reduction of root growth along with an impairment of root function due to which diminishes the nutrient-acquisition capacity of root systems. Reductions in nitrogen accumulation in root nodules and decrease in carbon and oxygen fluxes under drought conditions ultimately impede nitrogen fixation in legume crops. Drought may alter the soil microbial activity and composition, e.g., reduction of soil-nitrifying bacteria.

Excessive precipitation may cause significant soil nutrient loss like nitrate leaching. Poorly drained agricultural soils receiving episodes of frequent and/or intense precipitation may get waterlogged that become hypoxic (Otero, Figueroa, Muñoz, & Peña, 2011). Under low oxygen, any change in soil redox properties can give rise to elemental (Mn, Fe, Al, and B) toxicities which reduces crop yields, and further production of phytotoxic organic solutes impairs root growth and function. Hypoxia may also lead to nutrient deficiency; reason being the adenosine tri phosphate (ATP) synthesized via oxygen-dependent mitochondrial electron transport chain drives the active transport of ions into root cells. Hypoxic conditions may cause significant nitrogen losses through denitrification, as microorganisms use nitrate as an alternative electron acceptor in the absence of oxygen.

Effect on Biological Properties of Soil

Soil flora and fauna are indispensable components of all soil types which are known to play vital role in the incorporation, retention or breakdown of plant remains, biogeochemical cycling of nutrients, and their impact on soil porosity and structure. Global warming may not affect ecological composition directly, as soil flora and fauna share comparatively broad temperature optima. Moreover, any change in ecosystem and/or migration of vegetation zones can seriously influence less migratory soil fauna and flora due to increase in temperature and changes in rainfall pattern. Enhanced level of CO₂ in the atmosphere leads to enhancement in plant growth which in turn improves allocation of carbon below the ground, rendering the microbial population to accelerate the rate of nitrogen fixation (nitrogen immobilization as well as denitrification), increased soil aggregation, mycorrhizal associations, and weathering of minerals (Classen et al., 2015; Karmakar, Das, Dutta, & Rakshit, 2016).

Effect on Soil Erosion and Sediment Transport

No linear relationship exists between average annual rainfall, surface runoff, and extent of erosion/denudation. Combined influences of relief, climate (quantity and intensity of rainfall) and vegetation (type, density, continuity),

and erodibility characteristics of soil has a profound effect on the rate, type, and extension of soil erosion. Potential climate changes may influence soil erosion in either one of the two following ways:

- Intensive precipitation and thunderstorms may increase the rate of erosion due to higher runoff, if not counterbalanced by the soil conservation effect owing to greater dense and permanent vegetation because of better water supply.
- Lower rainfall may increase erosion due to wind. Overall lower precipitation may reduce the rate of erosion but may be balanced by the poor vegetation due to moisture limitations.

Effect on Availability of Soil Water

Climate-change drivers may affect the availability of soil water, e.g., precipitation may bring about rapid changes in soil water due to lesser time period for response, generally within a few hours; rise in temperature induces faster and more evapotranspiration thereby resulting in loss of water from the soil and finally land use type and pattern. The cumulative impact of climate—hydrology—vegetation—land use changes are portrayed by the soil moisture regime and field water balance. There is a projection that the availability of soil water in most of the regions of tropical countries shall decrease due to climate change. A severe decreasing trend in the precipitation pattern may influence dry parts of countries especially around Mediterranean Sea. The recession of water bodies would result in a decline in availability of water for irrigation purposes, as well as a reduction in fish production. Whole economies are likely to suffer when large water bodies recede ([Tripathi et al., 2016](#)).

Effect on Salinization and Alkalization

One of the expected consequences of global warming is rise in the level of Eustatic Sea: increase in the number of inundated territories (significantly around delta regions and river valleys, which happen to be densely populated) and the areas susceptible for sea water intrusion. Increased incidence of salinization and alkalization can occur in regions where either evaporation is increased or rainfall decreased. In sodic soils, increase in capillary rise increases transient salinity, bringing salts closer to the root zone. Episodic rainfalls may confine leaching due to surface sealing. This leads to an increase in subsoil drying which further increases the salt concentration in the soil solution. The areas where salinity is a consequence of recharge processes, salinization increase with an increase in upstream recharging rainfall ([Elliott et al., 2014](#)).

Effect on Acidification

A decrease in precipitation may hinder both downward filtration and leaching. The dominant vegetation types, their productivity, rate of decomposition, and

litter deposits are all determined by the climate which eventually indirectly influences soil reaction (Pankova & Konyushkova, 2013).

Effect on Incidence of Pests, Weeds, and Diseases

It is likely that an increase in temperature may prove conducive for proliferation of pests specifically detrimental to crop and livestock production (Sharma & Prabhakar, 2014).

EFFECT OF CLIMATE CHANGE ON FACTORS INFLUENCING AGRICULTURE

Climate change can affect agricultural production in numerous ways. The climate-change drivers can be roughly grouped into six categories, which are described under the following heads:

Temperature

Temperature fluctuations may affect water supplies, plants, animals, and incidence of pests. Any change in temperature may directly affect crop growth rates, incidence of pest, appetite and performance of livestock, and supply of water in reservoirs and soil.

Precipitation

At any point of time, rainfall may alter the direct availability of water to crops, the onslaught of drought stress on crops, the forage supply for animals, conditions for animal production, supply of irrigation water, aquaculture, and river flows affecting barrage transport.

Changes in Atmospheric CO₂

Atmospheric CO₂ influences growth of crop plants and weeds by changing one of the primary inputs for photosynthesis (Stocker & Intergovernmental Panel on Climate Change, 2013).

Extreme Events

Disasters and extreme events profoundly influence production conditions, may destroy trees or crops, immerse livestock, affect water supplies, and sway water-borne transport and ports. Sea-level upsurge may affect the expedience of ports for water-borne transport, immerse-producing lands and may impact aquaculture production conditions (Mainuddin et al., 2011).

CLIMATE CHANGE: IMPACT ON AGRICULTURE

The coherent impact of climate change is likely to increase crop yields (with sunflower, sugar beet, and winter wheat) probably due to the cumulative effects of longer growing seasons, radiation-use efficiency, and CO₂ fertilization, which is mostly applicable to species having C₃ photosynthetic pathway, whereas not necessarily in species sharing C₄ pathway. Elevated CO₂ levels have been found to increase both size as well as the dry weight of most C₃ plants including plant components (Rosenzweig et al., 2014; Tripathi et al., 2016).

During vegetative development, relatively more photo assimilate is known to be partitioned within structural components (e.g., petioles and stems) so as to underpin the light-harvesting apparatus (leaves). With increasing CO₂ concentration and temperature, the harvest index has a tendency to decrease. When grown under water-stressed-light-textured soils, increased yields could be expected for sunflower, however, small increase or possible decrease in yield for oilseed rape, potatoes, and high-quality horticultural crops. Increase in grass yield is also not ruled out. Climatic warming and increased levels of CO₂ in the atmosphere will enhance growth of trees in the short time span (Krishnan, Swain, Chandra Bhaskar, Nayak, & Dash, 2007).

Evidently, the tropical arid and semiarid areas, where crops have attained their maximum level of tolerance, it is plausible that crop yield will decrease owing to an increase in temperature. The reduction embosoms forest and its products and rangeland. Soil fertility of arable land suitable for agricultural production will diminish in traits, properties, and characteristics. Climate sensitivity could be extended to other agriculture sectors too, e.g., fisheries and poultry. Overall impact can be observed on production processes for food, feed, fiber, beverages, energy, industrial crops, livestock, poultry, fish, or forest.

Impact on Crops

Crops grown in any province are critical for the global food supply. Any changes in temperature, carbon dioxide (CO₂) levels, and extreme weather conditions may profoundly impact crop yield (Fig. 7.1). On the one hand, warmer temperatures may increase crops growth, but the same temperature could induce reduced yields. Crops have tendency to grow faster under warmer conditions. However, in some crops like grains, faster growth reduces the amount of time required by seeds to grow and mature which can reduce yields (i.e., the quantity of crop produced from a given amount of land).

For a particular crop, any impact of increased temperature is known to depend on the specific optimal temperature required for growth and reproduction of the crop. Although in some areas, an increase in temperature may benefit the kind of crops that are typically planted there, but at the same

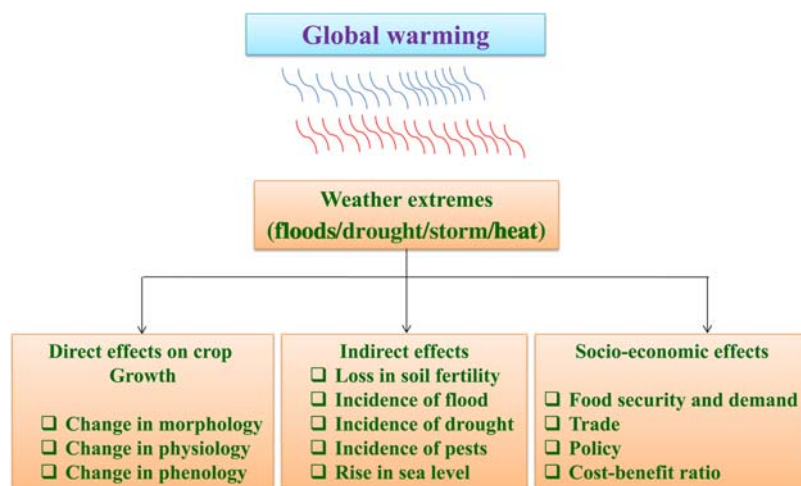


FIGURE 7.1 Effect of climate change on agriculture.

time, if warming overshoots a crop's optimum temperature, yield decline cannot be ruled out. Some of the possible impacts of climate change on agricultural crops are described under the following sections.

Impact on Wheat

Wheat cultivation occupies largest land area than any other commercial crop around the world. The cultivation of wheat extends from tropical to subtropical zones. It is an important staple widely used for food and fodder. Wheat can be cultivated in diverse conditions as it possesses high adaptability to adverse climatic conditions.

Availability and sustainable use of water reserves is main influencing factor during changed climatic conditions marked with frequent occurrence of extreme events like drought and inadequate groundwater recharge (Elliott et al., 2014). The impacts of climate change on wheat production are un conspicuous as it is unclear whether it will affect wheat production positively or negatively. The root cause of this outcome is opposing effects of precipitation, temperature, and concentration of CO₂ on metabolic processes of wheat growth. Carbon dioxide fertilization effect is projected to accelerate more primary production, whereas elevated temperature can retard net carbon gain due to increased respiration of the plant more than photosynthesis (Högy & Fangmeier, 2008). At elevated concentration of CO₂, overall decrease in micro- and macronutrients in wheat has been reported (DaMatta, Grandis, Arenque, & Buckeridge, 2010). In wheat, decrease in Mg, Ca, S, and N content has been reported at elevated CO₂, whereas P and K content have been found to change (Högy & Fangmeier, 2008). Leaf-protein concentration in

wheat may be decreased under increased CO₂ concentration (Altenbach, 2012). Induced water stress led by decrease in humidity, strong winds, and intense light may result in average granule size in wheat, affecting over all yield of crop (Hurkman & Wood, 2011).

Structure and gelatinization process of wheat starch may get affected by increase in temperature. The quality of grain might be affected due to slight change in temperature from 2 to 4°C, more than twice the effect of CO₂, as starch grain size, starch content, and gelatinization may be changed in complex manner with increase in temperature (Hurkman & Wood, 2011). Reduced winters, reduced winter fog, and winter rains have been found to affect the quality of the wheat produced by farmers in different parts of the world (Tripathi et al., 2016). In conclusion, climate change is playing crucial role in altering different biochemical and physiological processes of wheat growth and development, thereby affecting overall crop yield.

Impact on Rice

Rice is widely cultivated cereal around the world. In Asian subcontinent, more than 90% world's rice is produced. As per some crop yield simulation models, elevated concentration of CO₂ has been found to improve the yield of the rice due to its fertilization effect, whereas increased temperature may hamper rice production (Krishnan et al., 2007). An increase in the concentration of CO₂ improves the water-use efficiency of the paddy crop (Shimono, Suzuki, Aoki, Hasegawa, & Okada, 2010). According to some reports, with increase in global mean temperature, rice yield has been found to decrease (Streck, Bosco, & Lago, 2008). At increased concentration of CO₂, the shortening of the flowering period has been observed (Streck et al., 2008). Increased atmospheric temperature results increase in leaf surface temperature, thereby affecting primary production (Poudel & Kotani, 2013). Under increased CO₂ concentration in atmosphere, glucose content in foliage of rice improves which makes the foliage more susceptible for insect attack (Shimono et al., 2010). Enhanced respiratory losses and reduced grain filling duration have also been observed under aforesaid conditions (Kim et al., 2011). Development of panicle and meiosis is affected at increased temperature leading to anomalous pollen maturity and complete sterility (Endo et al., 2009). Thorough investigations of impact of climate change on rice production assert that the physiological processes of the plants are more affected. Increase in atmospheric temperature affects growth of the plant during different stages of growth and development.

Impact on Maize

Globally, maize serves as main starch resource and occupies more than 80% of the global starch market. Maize is cultivated on 100 million hectares of land in lower and middle income nations. Increased global mean temperature

coupled with reduced rainfall is supposed to affect maize production negatively. It may reduce yield by hampering all stages of maize growth and production in general and the stage of reproduction in particular which is most susceptible to increased temperature and water deficit conditions. As per the results of more than 20,000 historical maize field trial carried out in Africa, the grain yield of maize reduces by 1%–1.7% on increase of every degree above 30°C under optimal rain-fed and drought conditions.

The temperature increase beyond 32°C leads to decrease in yield of the grain particularly in tropical and moderate zones. Protein synthesis during seeding and other vegetative stages of the maize is affected by increased atmospheric temperature. Heat stress results in change in quality of the starch, reduction in granule size, and shortening of branch chains and maize yield (Wang & Frei, 2011).

At elevated temperature, viability and in vitro germination of pollen, fertilization, pollen water potential, the quality of pollen shed, and germination of pollen tube have been reported to be reduced (Lu et al., 2014). Expansion of maize cultivation led by climate change will turn new areas susceptible to spread of diseases (Wheeler & von Braun, 2013).

Impact on Fruit and Vegetable Crops

Globally, the demand of vegetables and fresh fruits is increasing day by day. Extensive research attempts have been made to assess the impact of climate change on physiological processes (respiration, photosynthesis, water relations in cell and membrane permeability and reproduction) of fruit and vegetable crops. It has been found that the stage of conversion of flower into fruit is directly affected by the climate change (Wheeler & von Braun, 2013). Climate change affects initial, early, and full flowering phenological stages of these crops (Ashebir et al., 2010).

High temperature reduces the production of plant hormones, primary and secondary metabolites. It also reduces the rate of seed germination in fruit and vegetable crops. Climate change led ultra violet (UV) effects has been found to result in production of anthocyanin which results alterations in genetic set up of vegetable and fruit crops.

Impact on Fisheries

Fisheries across the globe annually harvest several billion metric tonnes of fish, shellfish, etc. and contribute significantly to the economy. Already many fisheries are facing multiple stresses, which include water pollution and overfishing. Climate change may exacerbate these stresses (Brander, 2015; Chan et al., 2011; Cheung, Watson, & Pauly, 2013; Holbrook & Johnson, 2014; Portner & Peck, 2010). Change in temperature may lead to significant impacts which are as follows:

- The diversity of many species of fish and shellfish might change. Several marine species have specific range of temperature for survival, e.g., Sea cods in North Atlantic Ocean thrive well at water temperatures below 54°F. Even a small change in sea-bottom temperatures (around 47°F) can impair their capacity to reproduce and may affect the survival of young cods.
- Warmer climate may drive aquatic species toward colder areas of lakes and streams, or they may move northward in the ocean or along the coast.
- Some temperature sensitive diseases known to affect aquatic life are likely to become more dominant in warm water, e.g., in southern New England, a dramatic decline has been observed in lobster catches attributed to the onslaught of a temperature-sensitive bacterial shell disease responsible for the massive die-off events leading to the decline thereof.
- Any alteration in temperature and seasons is likely to influence the timings for reproduction and migration.

Impact on Livestock

Annual global consumption of meat is around 41.90 kg per person per year 2013 FAO. Apart from human consumption, livestock industry produces several billion dollars' worth of goods. Any changes in climate may affect animals as well as economy both directly and indirectly (Ghahramani & Moore, 2016; McKune et al., 2015; Ozkan et al., 2016).

- Under climate change, heat waves, which are likely to increase, may threaten livestock directly. It is reported that just one heat wave can create a loss of more than 5000 animals. Heat stress may influence animals both indirectly and directly. Progressively with time, heat stress can enhance susceptibility to disease, reduction in fertility, and reduction in production of milk.
- Drought under climate change may imperil pasture and feed supplies through retracting the measure of quality forage available for the grazing livestock.
- It is likely that climate change may enhance the invasion of pests and the diseases, parasites, and insects may significantly affect livestock. Warmer winters and the untimely inception of spring may consequently result in easy survival of certain parasites and pathogens. In areas receiving increased precipitation, moisture-reliant pathogens are likely to flourish.
- Elevation in carbon dioxide (CO₂) levels may increase the pasture productivity but may also decline their quality. Increase in CO₂ levels in the atmosphere may increase plant productivity on which livestock feed.

Effect of Increment of CO₂ and Temperature on Crops

It is conjecturable that higher CO₂ levels will stimulate photosynthesis in some plants; an approximate 30%–100% increase in photosynthesis rates could be achieved due to doubling of CO₂ levels. Laboratory scale

experimentations affirm that when more carbon is absorbed by plants, they tend to grow in size with a faster pace. This is specifically veritable for C_3 plants (the product of first biochemical reactions under photosynthesis has three carbon atoms). An increased level of CO_2 is known to suppress photorespiration in C_3 plants rendering them more water efficient. Major midlatitude food staples include crops such as wheat, rice, and soybean which are C_3 plants (Van der Kooi, Reich, Löw, De Kok, & Tausz, 2016). On the other hand, response of C_4 plants (the product of first biochemical reactions under photosynthesis has four carbon atoms) is not likely to be as climactic (in spite of the fact at present CO_2 levels the photosynthetic efficiency of these plants is more, as compared to C_3 plants). Several low-latitude crops such as sugarcane, maize, millet, and sorghum, as well as several pasture and forage grasses are included under C_4 plants.

Crop Responses to CO_2

Effect on Photosynthesis

In principle, any increase in atmospheric CO_2 concentration above present levels may increase the rate of photosynthesis by decreasing photorespiration (O_2 fixation instead of CO_2 by Rubisco), which is known to rise with temperature and happens to be higher in C_3 than C_4 and crassulacean acid metabolism plants (Sage, Christin, & Edwards, 2011). In addition, increase in CO_2 levels usually stimulates C_3 plant photosynthesis more than C_4 . Any doubling of the existing atmospheric concentration of CO_2 has been reported to stimulate the growth of C_4 plants by 10%–20%, whereas it has resulted stimulation of the growth of the C_3 plants to approximately 40%–45% (Ghannoum, Caemmerer, Ziska, & Conroy, 2000).

Effect on Respiration

Increase in the concentration of CO_2 may alter different metabolic processes in plant including cellular respiration. It has been underlined that; the elevated concentration of CO_2 reduces the rate of respiration in C_3 plants, thereby resulting in increase of plant biomass. On the other hand, the impacts of elevated concentration of CO_2 on C_4 plants have not been reported extensively to cause significant changes in respiration (Valeria & Santiago, 2011).

The effectiveness with which elevation in CO_2 can be translated into growth benefits depends on the balance of sink source and is influenced by different environmental factors.

Changes in the Ratio of CO_2/O_2

Any change in gaseous composition of the atmosphere may have profound effect on metabolism of the plants. The impact of elevated concentration of CO_2 may alter the quality of leaves which serves as source of energy for

animals. Increase in C/N ratios and decline in leaf-protein contents are common in case of all types of leaves under increased concentration of CO₂. The decrease in leaf-protein contents and increase in C/N ratios under elevated atmospheric carbon dioxide entail reduction in food quality to herbivores. [Stiling and Cornelissen \(2007\)](#) investigated plant–herbivore interactions and reported that increased concentration of CO₂ results in lowering of nutrient contents in C₃ plants which hampers the growth of the herbivores surviving on leaves exposed to elevated concentration of CO₂ ([Stiling & Cornelissen, 2007](#)).

In contrast to C₃ species, C₄ grasses are low-nutrient-containing food sources than C₃ grasses, both in terms increased C/N ratios and protein content. Elevated concentration of CO₂ affects the abundance of C₄ and C₃ plants in general and grasses in particular. The increased concentration of CO₂ is supposed to castrate the quality of the food consumed by grazers on coarse scale (C₄ vs C₃) as well as on fine scale (carbon/nitrogen ratio, protein content) ([Valeria & Santiago, 2011](#)).

Effect of CO₂ Increase Along With an Increase in Temperature

Elevated concentration of CO₂ has been projected to result drastic change over in annual precipitation patterns with 20% reduction in levels ([Schiermeier, 2008](#)). At elevated concentration of CO₂, the rate of the opening and closing of stomata and transpiration are reduced which hamper the loss of the heat from the plant ultimately leading to increase in temperature of plant cells ([Valeria & Santiago, 2011](#)).

Thus, down the line, plants are supposed to undergo acute heat and drought stress, leading to negative impacts on biodiversity, ecosystem productivity and biodiversity ([Thomas et al., 2004](#); [Valeria & Santiago, 2011](#)).

With reference to changing environment, supposedly due to increase in atmospheric concentration of CO₂, the sensitivity of metabolic processes, like plant photosynthesis to each environmental variables including vapor-pressure deficit and soil salinity, low water availability, increased atmospheric temperature, etc., has not been addressed and reported extensively ([Valeria & Santiago, 2011](#)).

The response of the plants due to elevated ambient CO₂ concentration will affect the extent of sequestration of the carbon besides affecting the productivity of ecosystem in future. However, these affects will be distinct among C₄ and C₃ plants. Ambient rise in carbon dioxide concentration may have synergistic impact on plant photosynthesis. Plant photosynthesis is believed to be the most thermosensitive, and the negative impacts of heat stress have already been very well reported ([Berry & Bjorkman, 1980](#)).

During the process of photosynthesis under both dark (Calvin cycle) and light (electron transport) reactions, thermolabile intermediates are formed especially in Photosystem II during light reactions and Rubiscoactivase in

the Calvin cycle (Berry & Bjorkman, 1980; Heckathorn et al., 2004; Weis and Berry, 1988). Therefore, limiting processes regulating photosynthesis at increased temperature could be either reductions in the capacity of rubiscoactivase to maintain Rubisco in an active configuration or reducing capacity of electron transport to regenerate ribulose-1,5-bisphosphate.

EFFECT OF FARMING ON CLIMATE CHANGE

Agriculture is highly exposed sector to climate change, as farming operations heavily rely on climatic conditions. Agricultural activities also contribute significant amount of GHGs to atmosphere. Methane (CH₄) is produced from livestock digestion processes and stored animal manure, whereas nitrous oxide (N₂O) from organic and mineral nitrogen fertilizers.

However, agriculture can also help to provide solutions to the overall climate change problem by reducing emissions and by sequestering carbon while not threatening and compromising viable food production.

ADAPTATION STRATEGIES TO MITIGATE CLIMATE CHANGE IMPACT ON AGRICULTURE

In agriculture sector, adaptive measures (both at sectorial and farm level) range from technological solutions to adjustments in management of the form or structures, and to political changes, such as adaptation plans (Burney et al., 2014; Matsui, Kobayasi, Kagata, & Horie, 2005). Focusing on farm and sectorial level adaptation, best possible short-to-medium-term adaptive strategies may involve the following.

Adaptation Strategies at Farm Level

Adjusting the Timing and Technicalities of Farm Operations

At farm level, adjustment in timing of farm activities may be very effective strategy to neutralize the effect of climate change. It may involve changing the dates for sowing, plantation, and other treatments (Yohannes, 2015). In Philippines, farmers widely adapt to the early onset of rainy season by performing cultivation on upland farms. It results in high agricultural yield in that season to the farmer leading to significant financial gains. Similar practices are being followed by the farmers of Tanzania. Shifting of date of plantation is routine practice in some West African countries like Niger, Burkina Faso, and Senegal, who follow a well-developed mathematical model for the cultivation of wide spectrum of crops to overcome the effect of adverse climate (Yohannes, 2015). Adjustment in date of plantation immensely helps the farmers of East Gojam (Choke Mountain in Ethiopia) and Eastern Hararghe, respectively. At farm level, ergonomic solutions, such as

improving animal shelters in terms of ventilation and cooling as well as protection of plantations and orchards from frost damage.

Employment of Climate-Resilient Crops and Varieties

The employment of climate-resilient crop varieties with improved resistance toward drought, flood, heat, and salinity stress is required to sustain good crop yield as well as to neutralize the adverse effects of climate change (Mall, Gupta, & Sonkar, 2017). It is necessary to bridge the yield gap, improve profitability and productivity for the sustenance of livelihood of millions of the people who depend on agriculture. Change in crop varieties may result in cryptic or more aggressive rooting habit, which favors better use of available soil moisture and permits greater soil-moisture storage under irrigation. Climate-resilient crops may not require greater demands of water and extended growing period (Mall et al., 2017). In order to minimize risks of climate change in rice-growing ecosystems, International Rice Research Institute has developed 28 new high yielding, salinity and drought-tolerant varieties. Developed rice varieties include cold-tolerant rice for Mali and Senegal, rice for Gambia, and iron-tolerant rice for Ghana, Guinea, and Burkina Faso. According to International Crops Research for Semi-Arid Tropics, hybrid crop varieties perform twofold better under temperature and drought stress.

Management of Fertilizers

Water and nitrogen are two most important limiting factors of optimum plant growth. Nitrogen-use efficiency around the world has been reported to be as low as 30%–40% entailing that the remaining 60% is returned to aquatic water bodies and atmosphere. In atmosphere, N_2O results global warming with other GHGs (Stuart, Schewe, & McDermott, 2014). In order to ensure high crop yield, global nitrogenous fertilizer demand is expected to increase by 60% by 2025. Under such circumstances, improvement in nitrogenous fertilizer-use efficiency by growing crops is supposed to mitigate GHG emissions by reducing emission of N_2O (Udvardi, Brodie, Riley, Kaeppler, & Lynch, 2015).

The potency of small doses of fertilizer will be improved if efficacy of nitrogen uptake can be improved. It is a fine example of a “no-regrets” adaptation strategy aimed to effective adaptation strategy of climate change offering ecological and production benefits to the concerned farmers.

Efficiency of fertilizer can be improved by following ways:

- Application in appropriate time, especially with respect to irrigation scheduling.
- Uniform application according to necessity/deficiency depending on soil and plant status (precision farming approach).
- Split dressings during appropriate stages of the plant growth.

- Placement within the soil.
- Employment of drip irrigation and microsprinkler.
- Employment of slow-release granules.

Management of Water

Effective water management at farm level involves management of irrigation and conservation of moisture of the soil. Improvement in moisture content of the soil and storage of water offers better crop performance under drought periods between rain fall or between irrigation (Chartzoulakis & Bertaki, 2015; Iglesias & Garrote, 2015; Olmstead, 2014). It also helps in better crop growth in advanced growth phases of rain-fed crops growing under inadequate rainfall. Water-management practices at farm level include the following:

- Employment of effective technologies of irrigation reduces unproductive loss of water by evaporation, e.g., sprinkler, rain guns, center pivots and linear move sprinklers, and drip methods of water application.
- Reduction of evaporation of stored water at farm.
- Uniform irrigation to reduce addition to saline water table in arid and semiarid areas where saline groundwater table is a problem.
- Use of mulching.
- Employment of rain-water harvesting system for groundwater recharge.
- Sustainable use of fertilizers, soil amendments, and pesticides.
- Improved drainage.

Management of Livestock

In order to minimize the impacts of climate change, some strategies like breeding of livestock species which are acclimatized to local climatic conditions, alternative feed production technologies, and diversification of livestock types should be followed (Yohannes, 2015; Johannesen, Nielsen, & Skonhøft, 2013).

Strategies like planting of trees with a rationale to shelter livestock have been adopted by the Ethiopian farmers to avoid the risk of climate change. Farmers keep their animals at home during feed and water-scarce condition and feed them through cut and carry system. At the time of management of livestock waste, manure should be collected quickly to prevent emission of GHGs. Some innovative devices to store and remove livestock waste are being launched by some producers of livestock in South Asia and China. These devices dismantle biosolids in fermentation units established underground for the production of bioenergy for lighting, cooking, and energy for on-farm activities. Broken solids in fermentation units can further be employed as fertilizers to improve fertility of the soil. Management of livestock in this way may serve as effective adaptation strategy of mitigation of climate change.

Management of Pests

The changes in global climate has facilitated the growth and revival of incurable pests (like Cassava mealy bug, fruit flies, etc.) and vector-causing diseases in plants (e.g., whitefly, *Bemisia tabaci*; Thrips (many species), Aphids (several species), and plant hoppers). The biochemistry, physiology, biogeography, and population dynamics of insects have changed drastically due to climate change. Due to development of resistance toward chemical pesticides, pest control strategies are slowly shifting toward more ecologically sound, sustainable, and economically viable options (Chidawanyika, Mudavanhu, & Nyamukondiwa, 2012). Biological pest-control strategies offer such chance through parasitism or predation of pests. Use of biopesticides is widely recommended as an important component of sustainable integrated pest management programs under changed climatic conditions. Biological pest control can be categorized into three components, namely conservation, classical, and augmentation control. In conservation, biological control practices, survival, and activities of natural enemies of the pests are promoted at the cost of pest populations under target. For instance, ecological strips comprising specific noncrop plants can be purposefully cultivated as a food and over wintering shelters for local natural enemies of the pests affecting cereals, fruit orchards, and vegetable crops.

In classical biological control, natural enemies of the pests are collected from their area of origin and released in area where their host was introduced accidentally. In the United States, *Eustern opusvillosus* (Boheman), *Bangasternus orientalis* (Carpimont), *Chaetorellia succinea* (Costa), and *Urophora sirunaseva* (Hering) have been successfully employed to control alien yellow star thistle *Centaurea solstitialis* [L] causing damage to seed head (Gutierrez, Ponti, d'Oultremont, & Ellis, 2008). Augmentative biological control involves time-to-time release of large numbers of mass-reared natural pest enemies in order to supplement natural pest enemy populations (Collier & Van Steenwyk, 2004; van Lenteren, 2012).

Conservation of Soil and Soil Moisture

Farmers of several countries namely Senegal, Kenya, Niger, and Burkina Faso widely practice soil-conservation techniques to avoid the negative impacts of climate change on crops. Farmers in Kamenyanga and Kintinku select appropriate timing for different farm activities. They bury the lignocellulosic biomass in the soil to replenish soil organic matter and fertility. They burn the plant biomass for quick release of nutrients. Contour ridges are used as an effective strategy to reduce soil erosion favoring better root penetration and improved moisture conservation by the farmers in Tanzania. Local farmers in Burkina Faso and Senegal have strengthened their adaptive capacity for climate change by employing traditional fertilization and pruning techniques to increase plant densities in semiarid areas. Such practices help in

holding soils intact and slow down desertification. Zero tilling and mulching is widely preferred by the farmers of Sahel as an effective strategy to conserve water and carbon in soil (Nyong, Adesina, & Osman Elasha, 2007).

Adaptation Strategies at Sectorial Level

1. Assessment of requirements and opportunities for changing crops, varieties in response to climate trends, and identification of vulnerable areas and sectors;
2. Assistance to research in agricultural area and to experimental production targeting at selection of the crops and development of novel varieties best suited to changed environmental conditions; and
3. Adaptive capacity building by creating awareness and provisions of salient information and advice on management of farm operations.

Monitoring systems for the measurement of long-term response of agricultural lands are numerous, but integration across these systems is scanty (Stocker and Intergovernmental Panel on Climate Change, 2013). Existing transition and state models could be extended to incorporate knowledge of how agricultural lands and products respond to global climate change. Integration of many such models with existing monitoring efforts and plant developmental data bases could offer cost–incentive strategies which may enhance knowledge of regional climate change impacts as well as offer management options for ecosystems.

In addition, there are no easy and reliable means to accurately ascertain the mineral and carbon state of agricultural lands, particularly over large areas at present. A fairly cost-effective method of monitoring biogeochemical response to global change would be to sample ecologically important target species in diverse ecosystems.

CLIMATE CHANGE AND FOOD SECURITY

Climate change can shift suitability of the land leading to increases in suitable cropland in higher latitudinal regions and hampering of potential cropland at lower latitudes. A slight rise in ambient temperature (ranging from 1°C to 3°C) is anticipated to favor crop yields in temperate zones but have adverse effects in seasonally dry and tropical regions particularly in case of cereal crops. However, all regions are expected to face negative effects due to warming of the climate more than 3°C (Stocker and Intergovernmental Panel on Climate Change, 2013; Tripathi et al., 2016).

Food Security Risks Are Basically Local and National

Agricultural production across the globe can be maintained relative to the anticipated prevailing baseline levels over the forthcoming 100 years with

moderate change in climate (below a 2°C warming). However, effects at regional level would differ widely, and some countries may experience limited output even after they take adaptive measures. This conclusion takes into the notice the positive effects of CO₂ fertilization but not other possible effects of change in climate, including changes in agricultural soils and pests.

Vulnerable People at Risk

People poor in terms of trade, lack of access to technology and information, weak infrastructure, and armed conflict will face more difficulty to deal with the consequences of climate change on agriculture. In semiarid and arid regions, many of world's poorest areas rely on discrete agricultural systems and are at greatest risk. The human populations living in South, East and Southeast Asia; sub-Saharan Africa; tropical areas of Latin America; and some Pacific island nations are at great risk due to change in climate.

Impacts of Climate Change on Different Dimensions of Food Security

Food availability, food accessibility, food utilization, and food stability are the bedrock of the global food security.

In the words of World Food Summit of 1996, Food security prevails “when all people, at all times, have physical and economic access to plentiful, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy” (DaMatta *et al.*, 2010; Parry, Rosenzweig, & Livermore, 2005; Rosenzweig *et al.*, 2014; Sharma & Prabhakar, 2014; Tirado, Clarke, Jaykus, McQuatters-Gollop, & Frank, 2010; Wheeler & von Braun, 2013).

Impact of Climate Change on Food Availability

Generally, food availability is the most commonly used parameter of food security; it refers to the existence of plentiful quantities of food with significant quality, ensured by domestic food grain production or import. Domestic food grain production, the country's stock, flow of food aid and trade, including imports and exchanges, are covered under physical availability of the food. Environmental degradation led by global climate change reduces arable and suitable land for agricultural production. Less fertile soils, depleting water resources, inadequate precipitation, increased damage to agricultural crops by pest and diseases in crops, and livestock result in substantial decline in crop yield and productivity of the animals imposing negative impact on country's labor force (Cheung *et al.*, 2013; Dawson, Perryman, & Osborne, 2016; Parry *et al.*, 2005; Rosenzweig & Parry, 1994). Core

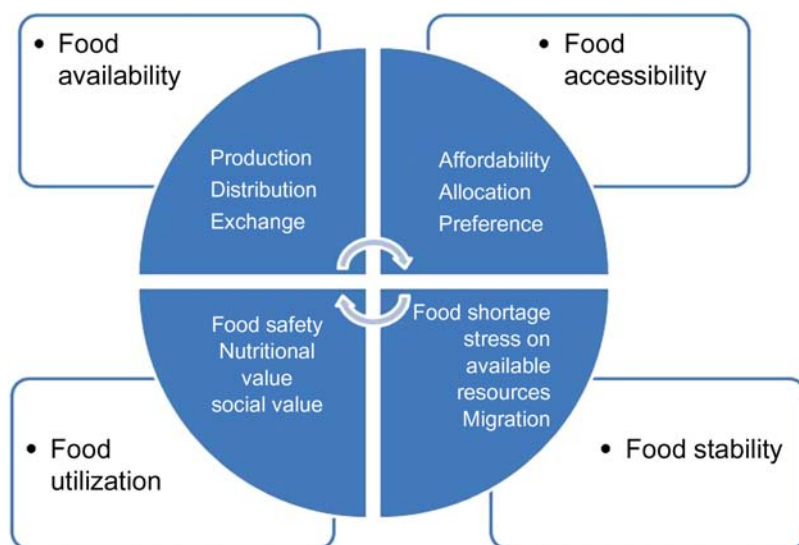


FIGURE 7.2 Core dimensions of food security under risk due to climate change.

Dimensions of Food security at risk due to climate change has been depicted in Fig. 7.2.

Impact of Climate Change on Food Accessibility

Ability of individuals, communities, and countries to purchase food in sufficient quantities and quality refers to food accessibility. Due to hike in food commodities, inaccessibility to markets, poverty, unemployment, weak educational backgrounds, and denial from property rights, households fail to access food in different parts of the world. Markets are only important as a secondary source of food for the population which depends on subsistence agriculture. Generally, there will be a hungry season, when yields of the crops do not meet demands, and food must largely be purchased from the markets. Decline in real prices for food and hike in real incomes over the past few years have resulted improvements in access to food in many developing countries in many folds. Possible hike in food price and reduction in the rate of income growth due to climate change may revert this trend (Dawson et al., 2016; Myers et al., 2014).

Impact of Climate Change on Food Utilization

The rate of production and pattern of different food items may be affected by global climate change thereby affecting nutritional requirements of the population.

For healthy life, the intake of diverse diet in terms of wide range of food sources like plant, animal or fish-based proteins, fresh fruits and vegetables, and staples are required. The nutritional aspect of food consumption generally refers to utilization component of food security. Food consumption patterns and nutrient contents of the food articles are essential components of food utilization. Individual or household capacity to consume and derive benefit from the food also refers to the food utilization. If a household who cannot get a nutritious and balanced diet, despite the fact that he has physical and economic access, could be food insecure. Poor people in several countries get essential micronutrients through consumption of green plants.

Climate change could directly affect micronutrient composition and consumption in several ways. It can affect micronutrient composition by altering agricultural yield or by changing the nutritional content of a particular crop, and/or by affecting plans to grow crops of different nutritional value (Bharucha & Pretty, 2010; Tirado et al., 2010).

Impact of Climate Change on Food Stability

All the other three dimensions mentioned above should be consistent over time and not be influenced adversely by social, economic, natural, or political reasons. Instability from climate change can arise due to increase in variability in agricultural production due to climate change (Stocker and Intergovernmental Panel on Climate Change, 2013; Tripathi et al., 2016).

Extreme events, like droughts, floods, and excessive high temperature at crucial periods in agricultural growth, exert pressure on stability of food access and utilization. Factors responsible for food insecurity are expected to get more prominent under the effect of global climate change culminating into frequent temporary food shortages, stresses on available resources' causing political unrests.

Movement of herdsmen and their animals due to climate change into new locations in order to search food and feed often give rise to conflicts. Climate change led conflicts over the issue of scarce resources like land and water, and drought-led migrations are mounting pressure on food security and nutrient availability to human population.

Policies for Improved Food Security

The adverse effects of climate change can be restricted by changing crops and their varieties, adapted tillage practices and planting schedules, improved irrigation systems and water management, better management of the watershed, and proper planning of land use.

In addition to dealing with the physiological response of plants and animals, policies can seek to enhance how the production and distribution systems cope with fluctuations in yields.

STRATEGIES TO MITIGATE IMPACT OF CLIMATE CHANGE IN AGRICULTURE AND FOOD SECURITY

The different adaptation strategies being formulated and developed at individual, organizational, and institutional level to avert the negative impacts of the climate change are as under ([Stocker and Intergovernmental Panel on Climate Change, 2013](#); [Tripathi et al., 2016](#)).

- Effective climate-smart agricultural production
- Livelihoods diversification/alternatives
- Resources decentralization under local governance
- Fossil fuel alternatives such as biofuels
- Infrastructure development
- Mass awareness toward climate change
- Natural hazard anticipation and early warning systems
- Insurance schemes and policy suggestions
- Research and development activities for development & dissemination of crop varieties suitably adapted to local areas
- Wise applications of genetic materials
- Vernacular knowledge use/gender considerations
- Promotion of integrated farming systems and agroforestry
- Improvement in infrastructure facilities: water harvesting techniques, storage and small-scale irrigation, etc.
- Improvement in water and soil management practices
- Adaptation to livelihood strategies and farming operation systems.

CONCLUSION

Climate change, the outcome of “Global Warming” has started to reveal its consequences worldwide. The primary determinant of agricultural productivity is “climate” which bears direct impact on global food production. Although life cycle of grain and oilseed crops are likely to progress more rapidly; but with increasing temperatures and variable rainfall, crops may start to experience failure, particularly under low and variable precipitation patterns.

Due to change in climate, northward shifting of plant species, range, and pastures, cropland weeds is adversely affecting livestock operations, grazing and crops. Increase in ambient temperature of the earth is likely to hamper production of the livestock during summer season. Ruminants are much more susceptible, as they are not provided shelter to overcome negative effects of the climate change.

As the climate of a particular country/region regulates the nature and properties of the crop and vegetation, agriculture sector is the more vulnerable to climate change. Duration of many crops including their respective yield is likely to be reduced due to increase in mean seasonal temperature.

However, the adverse effects of climate changes are low for economic activities including agriculture which ensure the availability and accessibility of food to people.

In the aforesaid backdrop, necessary way to combat food insecurity is to formulate and develop strategies facilitating availability of food through different agriculturally smart responses, improved and versatile livelihood sources. At the time of formulation and development of strategies to deal with the problem of climate change, requirements and aspirations of the susceptible people must be the guiding principle.

In order to prepare any nation for the climate change, a comprehensive knowledge of adaptation options across the complete range of warming scenarios, regions, and assessment of the impact of climate change on vulnerabilities of rain-fed agriculture sector would go long way.

A detailed and multipronged strategy of employing concerted research and development activities oriented to discover innovative technologies, vernacular coping mechanisms, and wider adoption of the existing technologies are required for adaptation and mitigation of climate change. In adoption of climate ready technologies in rain-fed agricultural areas, incentive of policies is supposed to play pivot role. A careful management of natural resources like water, biodiversity, and soil is required to cope with impact of climate change on agriculture. In context to Indian subcontinent, coordinated efforts at local, national, regional, and global level are required to combat climate change.

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Chapter 8

Impact of Climate Change on Livestock Production

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INTRODUCTION

Since the dawn of the green revolution, there has been progressive diversification toward livestock production within agriculture sector. As a component of agricultural sector, its share in the country’s gross domestic product (GDP) has been rising gradually, whereas that of crop sector has been on a decline. Though the contribution of agriculture and allied sector to the national GDP has declined during last few decades, the share of livestock to agricultural GDP had increased consistently. Livestock is, therefore, becoming increasingly unit in the growth of agricultural sector in the developing economies. Livestock contribute to socioeconomic development by augmenting increase and employment and reducing rural poverty. Livestock provide draught power and organic manure to the crop sector, and hides, skins, bones, blood and fiber are used in industries. Thus, livestock are an important source of income and employment, helping to alleviate poverty and smooth the income distribution among small landholders and the landless, who contribute bulk of rural population and the majority of livestock owners.

Climate change has the potential to be an increasingly formidable challenge to the development of the livestock sector. Global climate change, with predicted 1.5–5.8°C increases in temperatures by 2100 is likely to cause heat stress (HS) to create threats to animal productivity. Climate change has complicated impacts on animals affecting distribution, growth, incidence of diseases, availability of prey, productivity, and even extinction of species in extreme cases due to habitat loss. Both domestic and wild animals ranging from insects, amphibians, birds to mammals have been reported to be affected by global climate change, information on direct impact of climate change on animals is scarce (Nardone, Ronchi, Lacetera, Raniere, & Bernabucci, 2010). Most of the impacts of climate change are attributable to increased ambient temperature. Climate change has complex impacts on domestic animal production system affecting feed supply, challenging thermoregulatory mechanism resulting thermal stress, emerging new diseases due to change in epidemiology of diseases and causing many other indirect impacts. Global warming has two-way effects on animal production system. On the one hand, it directly affects the health, reproduction, nutrition, etc. of the animals resulting in poor performance, inferior product quality, outbreak of novel diseases, etc., whereas on the other hand, there are indirect effects on animal production due to change in soil fertility, decrease in preferred vegetation, rangeland degradation, desertification, and decrease in feed stuffs production. A vast number of studies illustrate the very diverse immediate effects of environmental factors and hence climate change on reproductive efficiency leading to alterations on genetic, phenotypic, and behavioral levels. Temperature and humidity, common environmental stressors are likely to increase in intensity due to the effects of climate change and can have significant impacts on growth, milk production, estrus expression, oocyte maturation, fertilization, and embryo development. Much of the effect of heat stress (HS) on establishment and maintenance of pregnancy involves changes in ovarian function and embryonic development that reduce the competence of the oocyte to be fertilized and the resultant embryo to develop. Decreased development of oocytes and/or embryos subjected to heat stress (HS) could be caused, at least in part, by heat shock-induced apoptosis, and alterations of the chromatin and spindle microtubules. Enhancement of thermo-tolerance of oocytes and embryos appears to be one of the solutions to early embryonic wastage. Discovery of novel genes responsible for compromised maternal recognition of pregnancy in endometrium and embryos will hint at new pathways and designing novel heat stress (HS) amelioration strategies for improving fertility/productivity of livestock species.

Measurement of Heat Stress Level

All animals have a range of ambient environmental temperatures termed the thermoneutral zone. This is the range of temperatures that are conducive to health and performance. The upper critical temperature is the point

at which HS effects begin to affect the animal. There are a number of environmental factors that contribute to HS. These include high temperature, high humidity, and radiant energy (sunlight). HS can be simply defined as the point where the cow cannot dissipate an adequate quantity of heat to maintain body thermal balance. Estimating impact of ambient conditions around animals on their performance has been done using the temperature–humidity index (THI) that takes into account ambient air temperature and humidity.

A. When ambient temperature is measured in °F (LPHSI, 1990):

$$\text{THI} = \text{db}^{\circ}\text{F} - \{(0.55 - 0.55 \text{ RH}) (\text{db}^{\circ}\text{F} - 58)\}$$

where db is the dry bulb temperature (°F) and RH = relative humidity (%) / 100.

Interpretation: If the calculated value is <72 = absence of HS, 72 to <74 = moderate HS, 74 to <78 = severe HS, and 78 and more = very severe HS.

B. When temperature is expressed in °C (Marai, Ayyat, & Abd El-Monem, 2001):

$$\text{THI} = \text{dbt}^{\circ}\text{C} - \{(0.31 - 0.31 \text{ RH}) (\text{dbt}^{\circ}\text{C} - 14.4)\}$$

where dbt is the dry bulb temperature (°C) and RH = relative humidity % / 100.

Interpretation: If the calculated value is <22.2 = absence of HS, 22.2 to <23.3 = moderate HS, 23.3 to <25.6 = severe HS, and 25.6 and more = extreme severe HS.

THI is usually classified into classes that indicate level of HS. However, definitions of those levels vary between indices and authors. McDowell, Hooven, and Camoens (1976) classified THI values into three classes: THI of 70 or less are considered comfortable, 75–78 stressful, and values greater than 78 cause extreme distress with lactating cows, 11 being unable to maintain thermoregulatory mechanisms or normal body temperatures. The Livestock Weather Safety Index classifications for HS are as follows: normal condition, $\text{THI} \leq 74$; alert, $74 < \text{THI} < 79$; danger, $79 \leq \text{THI} < 84$; and emergency, $\text{THI} \geq 84$. Du Preez, Giesecke, and Hattingh (1990) reported that dairy cow performance is affected, and cooling the animals becomes desirable, in the warning range of THI values 70–72. Milk production is seriously affected when THI values are 72–78, and the survival is threatened where THI values are 78–82.

Climate Change Impact on Growth

Climate change has significant effect on growth and production characteristics of animals. Growth, the increase in live body mass or cell multiplication, is controlled genetically and environmentally. The average daily gain (ADG) is influenced by factors like available nutrients, hormones, enzymes, and

environmental factors like increased ambient temperature (Padodara & Ninan Jacob, 2013). Exposure of sheep to elevated temperatures results in the decrease of body weight, ADG, growth rate, and body total solid, which is reflected by impaired reproduction (Marai et al., 2001). As per the crossbreds and buffaloes are affected more than indigenous livestock. As the crossbreds and buffaloes are more sensitive to temperature rise than indigenous cattle, a rise of 2–6°C due to global warming will negatively impact growth, puberty, and maturity of crossbreds and buffaloes, and attainment of puberty is delayed by 1–2 weeks. Under high ambient air temperature and solar radiation, steers reduce daily dry matter intake (DMI), causing a decrease in ADG, carcass weight and fat thickness (Mitlohner et al., 2001). Morand-Feher and Doreau (2001) reported a decline in feed intake ranging from 40% to 60% in 15-month-old buffaloes caused by variation of temperature and humidity from 21.5°C to 38.5°C and from 59 to 76.5 RH, respectively (Padodara & Ninan Jacob, 2013).

Exposure to hot environment had been found to negatively affect the growth of young calves. Nardone, Ronchi, Lacetera, and Bernabucci (2006) found lower wither height, oblique trunk length, hip width (–35%, –26%, and –29%, respectively) and body condition score (0.0 vs +0.4 points) in six 5-month-old female Holstein Friesian calves exposed to hot conditions as compared with a control group, kept under thermoneutrality conditions (Padodara & Ninan Jacob, 2013). Decrease in body growth and body reserves between birth and puberty, especially during the first few months was detrimental for milk production of the future cow and can increase the replacement rate later (Chillard, 1991). Animals exposed to chronic environmental stress undergo metabolic adaptations to elevate them (Padodara and Ninan Jacob, 2013) including changes in endocrine function, basal metabolism, metabolism of water and electrolytes, acid–base balance, and in ruminants an alteration in rumen fermentation. Rumen VFAvolatile fatty acid (VFA) production is increased in cold stress, due to increased feed intake and decreased HS. Selective forage intake during high environment temperature with alternative rumen fermentation caused a decrease in acetate and alters acetate: propionate ratio, which causes reduced milk fat yield during HS. High ambient air temperature had been observed to cause reduced appetite and growth in pigs. The heavier the pigs, the more they are affected. As protein deposits require more energy than fat deposits, the carcasses were leaner at slaughter (Xue, Dial, & Pettigrew, 1997). Compared to those reared in an optimal climate found that large white pigs reared in a tropical climate had a lower voluntary feed intake (–9%, –13%) and daily weight gain (–9%, –12%), leaner carcass, higher pH, lower moisture loss, and decreased lipid content of leaf fat in the entire backfat, concluding that tropical climate may have a favorable effect on pork quality (Padodara and Ninan Jacob, 2013). The adaptation in pigs to heat affects carcass characteristics by the reallocation of fat depots from subcutaneous sites (bardiere) toward innersites (panne) to facilitate thermal conductance (Le Dividich & Rinaldo, 1989).

Decrease in feed intake is one of the thermoregulatory physiological attempts of animals which decreased the metabolic rate, hence reducing metabolic heat production (Blackshaw & Blackshaw, 1994). Reduction in DMI indirectly helped to maintain core body temperature by reducing generation of heat during ruminal fermentation and nutrient metabolism. Feed consumption by dairy cattle starts to decline when average daily temperature reaches 25°C to 27°C (Beede & Collier, 1986), and voluntary feed intake was decreased by 10%–35% when ambient temperature reaches 35°C and above. Mallonee, Beede, Collier, and Wilcox (1985) reported that in hot weather, feed consumption by cows is reduced by 56% during the day time in outdoors as compared to cows kept indoors (Bajagay, 2011). In the same study, feed consumption was increased by 19% during the night time, and overall feed consumption was 13% less in cattle kept in outdoors as compared to cattle kept indoors. Decrease in DMI was more prominent in animals fed with roughage-based diet than in animals fed with concentrate-based diet. Similarly, reduction in DMI was more severe and rapid when food was poorly digestible. Decreased rumen motility due to thermal stress together with increased water intake resulted in gut fill which in turn reduced feed intake (Attebery & Johnson, 1969). Although digestibility of feed was reported to be increased at higher ambient temperature (Warren et al., 1974), absorption of nutrients from gastrointestinal tract is impaired during thermal stress (Bajagay, 2011). When ambient temperature is more than the normal body temperature, blood circulation to the skin and peripheral tissue increases with vasodilatation of peripheral blood vessels to transfer more heat from core to the skin surface and to hasten evaporative and convective heat loss from skin, thereby reducing blood supply to visceral organs including GI tract (Oakes, Walker, Ehrenkranz, Cefalo, & Chez, 1976). Reduction in intestinal blood flow may reduce the absorption of nutrients from the intestine (Bajagay, 2011).

Effect of Heat Stress on Milk Production

Climate can affect livestock both directly and indirectly (Adams et al., 1999; McCarthy et al., 2001). The direct effects are of weather and extreme events which could increase thermal stress for the animals and thereby affect animal health, feed efficiency, growth, reproduction rates, and milk production. Climate can also affect the quantity and quality of feedstuffs such as pasture, forage and grain, and the severity and distribution of livestock diseases and parasites. The indirect effects may result mainly from the impact of changes on livestock feed resources availability and price; the impact on livestock pastures and forage crop production and quality; changes in the distribution of livestock diseases and parasites. Other indirect effects are linked to the expected shortage of feed arising from the increasingly competitive demands of food, feed and fuel production, and

land use systems. Smit, McNabb, and Smihers (1996) attributed the indirect effects of climate-driven changes in animal performance to mainly alterations in the nutritional environment.

HS is a major factor that negatively affects the milk production in dairy animals. Decreased synthesis of hepatic glucose and lower nonesterified fatty acid level in blood during thermal stress causes reduced glucose supply to the mammary glands resulting low lactose synthesis which in turn ensues low milk yield. Reduction in milk yield is further intensified by decrease in feed consumption by the animals to compensate high environmental temperature. Actually, 35% of reduced milk production is due to decreased feed intake, whereas the remaining 65% is attributable to direct effect of thermal stress. Other factors resulting reduced milk production during thermal stress are decreased nutrient absorption, effect in rumen function and hormonal status and increased maintenance requirement resulting reduced net energy supply for production. HS reduced daily milk yield by 21% in Tunisia (Bouraoui, Lahmarb, Majdoubc, Djemalic, & Belyead, 2002). Mallonee et al. (1985) in Florida and Du Preez et al. (1990) in South Africa observed the same drop in milk production. However, a 10%–14% reduction in milk production was found when dairy cows were subjected to heat wave conditions associated with climate change in Argentina (Valtorta, Leva, Gallardo, & Scarpati, 2002). Du Preez et al. (1990) reported that milk yield decreased 10%–40% for Holstein cows in South Africa during the summer as compared to the winter. Frank, Mader, Harrington, Hahn, and Davis (2001) estimated reductions in milk production of 5.1% to 6.8% by 2090 in the Great Plains region. The milk yield losses were positively related with milk yield of cows (Berry et al., 1964). Johnson et al. (1988) found a higher average persistency decline in cows with milk yield higher than 30 kg/day compared with cows yielding less than 25 kg/day. The increase in milk yield increased the sensitivity of cattle to thermal stress and reduced the “threshold temperature” at which milk losses occurred. This is because metabolic heat production increased as the production level of a cow increased (Kadzere et al., 2002). Heat production of cows producing 18.5 and 31.6 kg/day of milk was 27.3% and 48.5% higher than nonlactating cows (Purwanto et al., 1990). Coppock et al. (1973) reported that high-producing dairy cows are affected more than low-producing cows, because the zone of thermal neutrality shifts to lower temperatures as milk yield, feed intake, and metabolic heat production increase. The stage of lactation is an important factor affecting dairy cows’ responses to heat. Johnson et al. (1988) observed that the midlactating dairy cows were the most heat sensitive compared to their early and late lactating counterparts. Midlactating dairy cows showed a higher decline in milk production (–38%) when the animals were exposed to heat.

Milk production is a function of THI. Milk production decreased as THI increased (Bouraoui et al., 2002). It is believed that milk yield began to decrease when the THI reached 72 and declined sharply when THI of 76 was exceeded (Johnson, 1985; Wiersma, 1990). Johnson, Ragsdale, Berry, and Shanklin (1962) showed a reduction in milk yield by 0.26 kg/day per unit if THI is more than 70. Johnson (1980) and Ravagnolo, Misztal, and Hoogenboom (2000) in Georgia defined $\text{THI} = 72$ as the threshold above which milk started to decrease. Ravagnolo et al. (2000) reported that maximum temperature and minimum relative humidity were the most critical variables to quantify HS, and both variables are easily combined into a THI. Milk yield declined by 0.2 kg per unit increase in THI when THI exceeded 72. West (2003) reported that of the environmental variables studied during hot weather the mean THI 2 days earlier had the greatest effect on milk yield, whereas DMI was most sensitive to the mean air temperature 2 days earlier. Milk yield for Holsteins declined 0.88 kg per THI unit increase for the 2-d lag of mean THI, and DMI declined 0.85 kg for each degree ($^{\circ}\text{C}$) increase in the mean air temperature. The decline in milk yield and DMI per unit of increase in the environmental measure was substantially less when evaluated on same day climatic measures in comparison with climatic measures 2 days earlier. Bohmanova, Misztal, and Cole (2007) stated that milk yield decreased by 0.23 to 0.59 kg per THI unit per day in the United States. Bohmanova et al. (2007) indicated a THI threshold of 72 in Georgia and 74 in Arizona. Zimbelman et al. (2009) indicated that the high-yielding dairy cows in Arizona reduced their milk yield at a THI of approximately 68. This is quite close to the results of Bouraoui et al. (2002) in Tunisia who reported that milk yield started to decrease at a THI value of 69. Dikmen and Hansen (2009) reported a THI threshold of 78.2 in Florida. Bernabucci et al. (2010) reported a decrease of 0.27 kg milk per day for each THI unit increase above 68. In Germany, Brügemann, Gernand, König von Borstel, and König (2012) indicated that milk yield decline between 0.08 and 0.26 kg for each unit increase in THI unit above 60, depending on the region. Brügemann et al. (2012) identified THI thresholds of 60 for milk yield in Lower Saxony (Germany). The THI breaking point ranged from 73 to 76 in Italy (Bernabucci et al., 2010). The observed variation threshold may have occurred because of the methods used for detecting the THI 10 thresholds. In addition, the threshold of dairy cattle for heat tolerance depends on the genotype as well as production level (Johnson, 1987; Hahn, 1989).

Hot environment negatively affected milk quality (Bernabucci and Calamari, 1998). Above 72 THI value milk protein content declined, whereas the response of fat yield seems delayed, and results were very contradictory. On comparing milk production during summer and spring in a dairy herd located in central Italy, a lower milk yield (-10%) and lower casein percentages and casein number in summer (2.18% vs 2.58% and 72.4% vs 77.7%,

respectively) were observed (Bernabucci et al., 2002). The fall in casein was due to the reduction in α s-casein and β -casein percentages. No differences were found between the two seasons for κ -casein, α -lactoalbumin and β -lactoglobulin, whereas serum protein contents were higher in summer than in spring. The strict relationship between casein content and fraction and milk behavior during technical processes can explain the loss in cheese yield and the alteration of cheese-making properties during summer in Italy.

Effect of Heat Stress on Estrus and Estrous Cycle

Thermal stress is one of the greatest climatic challenges faced by the domestic animals, and global climate warming may further aggravate the condition and even provoke new episodes of thermal stress condition. Seasonal variation of environment, nutrition, and management alters estrous activity and duration of estrus. In times of HS, estrus is reduced in duration and intensity. Cows exposed to high-temperature conditions may become physically lethargic. Therefore, a cow is less likely to exhibit standing estrus and other behaviors associated with estrus during peak temperatures. Cattle are more prone to exhibit signs of estrus during cooler temperatures, which predominately occur at night. With these reductions and changes in estrus behavior, producers have fewer opportunities to witness their cattle in estrus. Dairy cows in estrus during the summer displayed only 4.5 mounts per estrus compared to 8.6 mounts per estrus displayed in the winter. In summer, motor activity and other manifestations of estrus are reduced, and the incidence of anestrus and silent ovulation are increased. These effects lead to a reduction in the number of mounts in hot weather compared to cold weather, leading to poor detection of estrus. Therefore, in hot climates, there is a reduction in the number of inseminations and an increase in the proportion of inseminations that do not result in conception.

HS can cause an increase in cortisol secretion, which has been reported to block estradiol and reduce estrus behavior. Cortisol increases the concentrations of progesterone in blood resulting in negative feedback on the hypothalamus, which decreases GnRH, and in turn, reduces LH and estradiol concentrations in blood. Due to low levels of LH and estradiol, there will be no estrus or ovulation. Increased corticosteroid secretion can inhibit GnRH and thus LH secretion. HS had been found to inhibit the secretion of gonadotropins to a greater degree in cows with low plasma concentrations of estradiol compared to those with high concentrations suggesting that high concentrations of estradiol can counteract the effect of HS, or alternatively, that the neuroendocrine mechanism controlling gonadotropin secretion is more sensitive to HS when concentrations of plasma estradiol are low. It has been suggested that HS could also act directly on the ovary to decrease its sensitivity to gonadotropin stimulation. Regardless of the precise mechanism, any alteration in the secretory activity of the follicle and perhaps the corpus

luteum caused by HS were important factors in summer infertility. In hyperthermia, adrenal function is reduced, and this may allow the animal to cope with the environment because of the lower calorogenic actions of glucocorticoids. Estrogens are lower during the proestrus to metestrus period of the estrous cycle and during late gestation and appear to manifest their physiological actions through shorter duration of estrus and lower calf-birth weights, respectively. HS had been observed to decrease blood flow to uterine and oviductal areas which may reduce nutrients and increase biochemical waste products at the tissue level. In the early embryonic stages of development, HSP production increases (Lucy, 30 Nov. 2006). The increase in HSP production alters the binding activities of steroid receptors. The reduction of the steroid receptors diminishes the action of steroid hormones, predominately progesterone and estrogen on the reproductive tract. The lack of these hormones may cause embryonic death during pregnancy.

The function of the corpus luteum is to produce progesterone required for maintenance of pregnancy. If pregnancy does not occur, estradiol is the hormone responsible for inducing luteolysis. Estradiol concentrations are reduced in heat-stressed animal, preventing the corpus luteum from regression. If luteolysis does not occur, the animal will remain in a sustained luteal phase because progesterone has a negative feedback on GnRH production. With the sustained presence of progesterone, estrus will not occur and neither will ovulation nor pregnancy. Furthermore, plasma estradiol concentrations are decreased in heat-stressed cattle. FSH and LH receptors are reduced on granulosa cells. LH concentrations are also reduced (Al-Katanani, Paula-Lopes, & Hansen, 2002). The number of follicles in heat-stressed dairy cows does not vary from nonheat-stressed cows. The main difference: follicles in heat-stressed cows are not ovulated because of the lack of recruitment in the first follicular wave. In order for follicles to grow and exhibit dominance, they first must have LH receptors on the granulosa cells (Wolfenson et al., 1995). A lack of LH receptors causes a decrease in the conversion of testosterone to estradiol. A lack of estradiol results in an increase in the number of subordinate follicles. These subordinate follicles will never gain dominance and therefore will not be ovulated. Follicular growth rate increases during the second follicular wave in heat-stressed dairy cows. As a result, heat-stressed animals enter the second follicular wave earlier than nonheat-stressed animals. Furthermore, aged follicles may be ovulated due to the increased duration of time the oocyte remains in the ovary. High temperatures can lead to a loss of pregnancy in heat-stressed dairy animals. HS at and immediately after the time of breeding results in lower conception rates. Heat-stressed dairy cows tend to have a decrease in DMI thus reducing the amount of energy in their diet. During times of HS, animals tend to cool themselves by blood vessel dilation near the surface of the skin in order to release heat. This results in decreased blood flow to the reproductive tract and reduces the efficacy of countercurrent heat exchange between capillary vessels.

Impact of Heat Stress on Oocyte Maturation

Elevated temperature is a major factor responsible for the reduced fertility in farm animals during the hot season in tropical areas. HS can compromise reproductive events by decreasing the expression of estrous behavior, altering ovarian follicular development, compromising oocyte competence, and inhibiting embryonic development (Hansen et al., 2001). During oocyte meiotic maturation, nuclear and cytoplasmic events must occur in a discrete spatial and temporal manner to acquire the capacity to be fertilized and undergo subsequent development. Several of these developmentally important processes are altered when oocytes are exposed to elevated temperatures during maturation. Exposure of the oocyte to HS during early stages of maturation (Edwards and Hansen, 1997) or at the germinal vesicle stage (Payton et al., 2004) results in deleterious effects on the internal organelles so that the capacity of the oocyte for fertilization and further embryonic development is reduced. Elevated temperature for the first 12 hours of maturation reduced de novo protein synthesis in bovine oocytes by approximately 40%, as measured by TCA precipitation, compared to controls. Exposure to an elevated physiologically relevant temperature during the first 12 hours of in vitro maturation has been found to hasten nuclear maturation (Edwards, Saxton, Lawrence, Payton, & Dunlap, 2005); in extreme cases, it may actually inhibit nuclear maturation. Heat-induced alterations at the cytoplasmic level are also evident and may include decreased de novo protein synthesis (Edwards and Hansen, 1996), altered cortical granule translocation (Edwards et al., 2005), calcium release (Tseng et al., 2009), and modifications of cytoskeletal components (Roth and Hansen, 2005). Although nuclear maturation seems to be minimally impacted by HS, several lines of evidence indicate that elevated temperature impacts certain aspects of oocyte cytoplasmic maturation. The cytoskeleton is mainly composed of microtubuli and microfilaments, and their structure was shown to be altered in mature bovine oocytes and two-cell bovine embryos exposed to temperatures of 41–41.5°C. The Golgi system and the endoplasmic reticulum become fragmented under stress conditions, and the number of mitochondria and lysosomes decreases which lead to a dramatic drop in adenosine tri phosphate (ATP) levels during HS. Many intracellular processes which are relying on these organelles are negatively affected by HS. Changes in membrane morphology were observed such as alteration in the ratio of protein to lipids and an increased fluidity of the membranes (Kruuv et al., 1983). These membrane changes which resulted in increased membrane permeability led to a drop in cytosolic pH and to changes in ion homeostasis (Coote, Cole, & Jones, 1991). Another possible mechanism that may be involved in disrupted cell function due to HS is apoptosis or “the programmed cell death.” Depending on the duration and severity of HS, these mechanisms ultimately lead to an arrest of the cell cycle and a stagnation of growth and proliferation and may result in the death of the

cell. Cellular exposure to HS may also induce oxidative stress. On the other hand, cellular exposure to HS increased the production of reactive oxygen species (ROS) and/or the promotion of cellular oxidation events. ROS such as superoxide anions (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2) can cause lipid peroxidation and enzyme inactivation, resulting in cell damage by promoting hydroxyl radical formation.

Direct exposure of bovine oocytes at the GV stage to an elevated temperature (41.8°C) for 12 hours reduced their ability to complete nuclear maturation and development after fertilization (Payton et al., 2004). Moreover, some studies have demonstrated DNA fragmentation and cytoskeleton disruption of oocytes after direct exposure to elevated temperature before or during maturation culture (Roth and Hansen, 2004). Roth and Hansen (2004) reported that elevated temperatures within the physiological range (40 – 41.8°C) during maturation culture increased programmed cell death in bovine oocytes. These observations suggested that the exposure of oocytes to elevated temperature induced DNA damage in oocytes prior to fertilization. They suggested that activation of apoptotic processes mediated by the group II caspases, caused by heat shock during oocyte maturation, was a critical mechanism responsible for the disruption of oocyte capacity for cleavage and subsequent development. When the oocytes were exposed to 41.8°C for 1 hour or more, more MII-stage oocytes had DNA-fragmented nuclei compared with MII-stage oocytes stored at 38.5°C . Moreover, the proportions of DNA fragmentation in total oocytes exposed to 41.8°C were higher than in those stored at 38.5°C , irrespective of the duration of treatment. The effects of exposure of oocytes to 41.8°C on the quality of oocytes were dependent on the exposure time. Ju and Tseng (2004) reported that abnormalities in the chromosomes, spindle microtubules, and pericytoplasmic microtubules of porcine oocytes occurred when the oocytes were exposed to 41.8°C for a short interval (1 hour). Furthermore, they suggested that these deleterious effects of hyperthermia on porcine oocytes were irreversible, even if the oocytes were returned to normal culture conditions. It has been demonstrated that mouse GV-stage oocytes exposed to 41.8°C reduced synthesis of intracellular proteins and that the heat-induced reduction of protein synthesis intensified as the duration of the heat shock increased. Heat shock during oocyte maturation promoted an apoptotic response mediated by group II caspases, which are responsible for the destruction of structural and regulatory proteins that lead to DNA damage and cell demise (Roth and Hansen, 2004). The effects of HS on fertility are dependent upon duration and severity of the stress imposed. Exposure of superovulated heifers to 42°C and 75% relative humidity for 10 hours at the onset of estrus has been found to raise respiration rates by more than 200% and rectal temperatures from 38.9°C to 41.3°C (Putney, Mullins, Thatcher, Drost, & Gross, 1989). This maternal HS was sufficient to increase the proportion of retarded embryos and reduce embryo quality 7 days after artificial insemination but did not alter the fertilization rate (Putney et al., 1989). Exposure of sheep to high temperature

and humidity (90–95°F and 60%–65% relative humidity) at the time of breeding increased the proportion of abnormal oocytes and prevented lambing (Dutt, 1963). As the duration of HS increases, the consequences become more pronounced. Specifically, mice exposed to 35°C and 65% relative humidity for 15.5 hours at the time of hCG administration experienced increased arrest of oocytes at metaphase I (MI) and increased oocyte degeneration and fragmentation (Baumgartner and Chrisman, 1981). Exposure of mice to 35°C and 65% relative humidity for 12.5 hours during oocyte maturation altered nuclear maturation by increasing oocytes arrested at MI and those with retained polar bodies; after mating, increased pre- and postimplantation embryo losses were observed with reduced fetal size and rate of development (Baumgartner and Chrisman, 1987). Improved embryo development after antioxidant treatment may be indicative of heat-induced increases in ROS in conjunction with HS conditions.

Impact of Heat Stress on Embryo Development

Embryonic development to the blastocyst stage and uterine differentiation to the receptive environment are crucial for successful establishment of the embryo–uterine crosstalk that leads to the initiation and progression of successful implantation. Environmental stress due to climate change has detrimental effect on the quality and developmental competence of oocytes. The embryonic developmental rate and hatched blastocyst rate reduced when embryos were exposed to high temperature *in vitro*. Heat shock caused a greater reduction in the proportion of cultured two-cell embryos that developed to the blastocyst stage than heat shock of four- to eight-cell embryos; morulae were unaffected by heat shock. Early embryos (<8- to 16-cell stage) would be more susceptible to heat shock because these embryos are transcriptionally quiescent and unable to produce protective molecules such as heat shock protein 70 (HSP70) in response to heat shock. Induction of heat shock proteins (HSP70) may enable embryos to tolerate the stress of an abnormal uterine environment by inducing thermotolerance. Embryonic loss can occur when there is disruption in the physiological regulation of embryonic development due to intrinsic errors in specific environmental stresses imposed on the mother. Whether or not an embryo can survive perturbations in the maternal environment depends on the degree to which the embryo can either (1) adjust its own internal physiology to allow for its survival and continued development or (2) act on the mother to restore to some degree the microenvironment that the embryo resides in.

Exposure to heat (39.5–41°C) during the first 48 hours of *in vitro* culture (IVC) of bovine zygotes significantly reduced the rate of development to the 8-cell stage at 72 hours of IVC and the rate of development to the morula or blastocyst stage at 144 hours of IVC (Sugiyama, McGowan, Kafi,

Philips, & Mary, 2003). Further, the reduction in the percentage of embryos that developed into blastocysts was especially notable in the heat treatment (41°C for 12 hours) at the 2-cell (Edwards and Hansen, 1997) and 1-cell stage embryos (Rivera and Hansen, 2001). Environmental stress has a lethal effect on the embryos both in vivo and in vitro. Mortality of preimplantation embryos increased when the pregnant mammals were kept under HS, and the conception rate increased when HS was mitigated in summer. In cattle, as for other species, exposure of pregnant females to HS during the embryonic period leads to embryonic loss (Putney, Drost, & Thatcher, 1988). Heat shock leads to embryonic death, at least in part, because protein synthesis is reduced (Edwards and Hansen, 1996; Edwards et al., 1997) and, at least in the mouse (Aréchiga, Ealy, & Hansen, 1995), free radical metabolism is increased. Heat-induced reductions in embryo development do not appear to be due to an inability of the oocyte to mature at the nuclear level. Exposure of bovine oocytes to 41°C during maturation reduced polar body extrusion (Lenz, Ball, Leibfried, Ax, & First, 1983). Edwards et al. (2005) reported that fewer heat-stressed oocytes had a discernible polar body at 24 hours, but a similar proportion was at MII, likely indicative of heat-induced polar body degeneration. Roth and Hansen (2005) reported reduced ability of oocytes to progress to MII after HS due to increased arrest at MI with misshapen meiotic spindles because of alterations in pH due to increased CO₂ concentrations. The maturation rate of the cytoplasm may also be altered by HS. During maturation, the arrangement of cortical granules, small membrane-bound secretory granules that undergo exocytosis to block polyspermy penetration, changes from an aggregated state throughout the cytoplasm (types I and II distribution) to a dispersed state along the oolemma (type III distribution). In bovine ova exposed to 41°C during the first 12 hours of maturation, there was a decrease in type II and increase in type III cortical granules compared to controls at 24 hours (Edwards et al., 2005) suggesting that the process of cytoplasmic maturation in heat-stressed ova may be faster than in controls. The nucleus concurrently undergoes changes when the egg is exposed to elevated temperatures during maturation. HS has been found to induce elevations in cytoplasmic ROS to reduce development. When exposure of maturing oocytes to elevated temperature was sufficient to reduce blastocyst yield by at least 20% compared to controls, addition of retinol, an antioxidant, prevented heat-induced reductions in blastocyst development (Lawrence et al., 2004). However, levels of the abundant and ubiquitous intracellular antioxidant, glutathione, were unchanged in heat-stressed in vivo matured murine oocytes despite heat-induced reductions in blastocyst development (Matsuzuka, Ozawa, Hirabayashi, Ushitani, & Kanai, 2004). Early embryos (<8- to 16-cell stage) are more susceptible to heat shock because these embryos are transcriptionally quiescent and unable to produce protective molecules such as heat shock protein 70 (HSP70) in response to heat shock. Induction of heat shock proteins (HSP70) may enable embryos

to tolerate the stress of an abnormal uterine environment by inducing thermotolerance. Elevated temperature for the first 12 hours of maturation reduced de novo protein synthesis in bovine oocytes by approximately 40% as compared to controls (Edwards and Hansen 1996). Exposure of GV-stage and matured (MII) murine oocytes to elevated temperature also resulted in reduced protein synthesis. Despite several studies examining the effects of elevated temperature on maturing oocytes, very little information is available regarding the effects of HS on molecular events occurring during meiotic maturation, fertilization, and embryo development.

Strategies for Mitigation

Genetic Approach

The unfavorable effects of HS can be mitigated by developing animals with improved thermotolerance using genetic approaches. Many local breeds are tolerant to extreme temperature, resistant to diseases and to survive, regularly produce/reproduce in low/poor management conditions and feeding regimes. Several studies have shown that *Bos indicus* embryos and embryos of the Romosinuano, a thermotolerant *Bos taurus*, are less affected by elevated temperature in culture than Holstein or Angus embryos. Identification of the genes controlling cellular thermotolerance will create the opportunity for transferring thermotolerance genes to breeds that are not adapted to hot climates. Block, Chase, and Hansen (2002) reported that embryos produced by insemination of Brahman oocytes with Angus semen were more thermotolerant than embryos produced by insemination of Holstein oocytes with Angus semen. There was no difference in thermotolerance between day-4 embryos produced by insemination of Holstein oocytes with Brahman semen versus embryos produced by insemination of Holstein oocytes with Angus semen. Genetic selection for thermotolerance and crossbreeding (Madalena, Lemos, Teodoro, Barbosa, & Monteiro, 1990), the local population with heat-tolerant breeds will help in minimizing the climate change impact on livestock productivity. There are also specific genes that could be selected which confer increased thermoregulatory ability, including those for coat color and the slick gene identified in Senepol cattle that causes short hair length. Selection for low rectal temperature might lead to indirect selection for cows with low milk production because of the negative association between level of milk yield and resistance to HS (Berman et al., 1985). One option is to select for production under the constraints of the environment the animals are reared in. In one study, selection for growth rate of cattle in a hot environment led to development of animals with increased thermal resistance. Certain tropically adapted breeds are also more resistant to elevated temperature at the cellular level. For example, heat shock killed a smaller proportion of lymphocytes from Brahman and Senepol cows than from Holstein and

Angus cows. Recently, it was demonstrated that the reduction in development caused by culturing embryos at 41°C for 6 hours was less for embryos from Brahman cows than for embryos from Holstein and Angus cows. Identification of the genes responsible for enhanced cellular resistance to heat shock may allow these genes to be transferred into thermally sensitive breeds through conventional or transgenic breeding techniques to produce an animal whose oocytes and embryos have increased resistance to elevated temperature.

Physical Modification of Environment

- The stressful effects of HS can be mitigated by protecting the cow from direct and indirect solar radiation. It was estimated that total heat load could be reduced from 30% to 50% with a well-designed shade, and shading is one of the more easily implemented and economical methods to minimize heat from solar radiation. Shades can be either natural or artificial. Tree shades have proved to be more efficient. When enough natural shade is unavailable, artificial structures may be constructed. Shades with straw roofs are best, as they have a high insulation value and a reflective surface. Uninsulated aluminum or bright galvanized steel roofs are also good. The best shades have white or reflective upper surfaces.
- Improved systems capable of either cooling the cow directly or cooling the surrounding environment are necessary to better control the cow's body temperature and maintain production in hot, humid climates. Evaporative cooling systems have been found to improve the environment in arid climates (Takamitsu, Takahashi, Kurihara, & Kume, 1987) and can be accomplished by passing air over a water surface, passing air through a wetted pad, or by atomizing or misting water into the air stream. Much of the water is needed for evaporative heat loss via respiration to help them cool off. Hence, provision has to be made for supply of continuous clean, fresh, and cool water to the animals. The single use of a sprinkling and fan system before milkings has proved to be useful to relieve dairy cows, HS, in terms of efficiency to reduce the impact of heat waves under a grazing system (Valtorta et al., 2002). Similarly, cows should be provided bedding and warmth to protect them from extreme cold.
- Increasing the air circulation in the animal sheds will help the animals in dissipating the heat, as it affects convective and, according to air humidity, evaporative heat losses. The air circulation inside the shed can be increased by keeping half side wall, i.e., open housing system, use of fan, increasing the height of the building.
- Climate change effect can be mitigated by diversifying farming practices. The use of heat-tolerant crop varieties such as sorghum and millet is another mitigation strategy. As the rainy season is short and ends earlier, early maturing plant/crop varieties could also be used as a mitigation strategy.

Nutritional Management

Nutritional modifications to account for changing nutrient requirements are necessary to adjust for the impact of HS due to reduced DMI and altered nutrient requirements.

- Water is arguably the most important nutrient for the dairy cow. Water intake increased by 1.2 kg/°C increase in minimum ambient temperature, but regardless of rate of increase, it is obvious that abundant water must be available at all times under hot conditions. [Milam et al. \(1986\)](#) reported that offering chilled drinking water enhanced milk yield for lactating cows by reducing body temperature through absorbed heat energy.
- Feeding dietary fat will provide extra energy during the time of negative energy balance. Incorporation of dietary fat at the level of 2%–6% will increase dietary energy density in summer to compensate for lower feed intake.
- Synthetic amino acids will reduce dietary crude protein levels. Excessive dietary protein or amino acids generate more heat during digestion and metabolism. Dietary protein degradability may be particularly critical under HS conditions. Diets with low (31.2% of CP) and high (39.2% of CP) rumen undegradable protein (RUP) fed during hot weather had no effect on DMI; however, milk yield increased by 2.4 kg/d and blood urea N declined from 17.5 to 13.3 mg/100 ml for the diet containing higher RUP ([Belibasakis, Ambatzidis, Aktsali, & Tsirgogianni, 1995](#)).
- HS-induced oxidative damage can be reduced by antioxidant defense mechanisms that protect the cells against cellular oxidants and repair system that prevent the accumulation of oxidatively damaged molecules. In vitro heat shock had been found to cause a reduction in the intracellular concentrations of the antioxidant glutathione in mouse morulae ([Aréchiga et al., 1995](#)) and that addition of various antioxidants to culture media, including taurine, glutathione, and vitamin E, provided some thermoprotection to mouse ([Aréchiga et al., 1995](#)) and cow morulae ([Ealy, Drost, Barros, & Hansen, 1992](#)). Supplementation of electrolytes (sodium, potassium, chloride) combats HS in various livestock species. Vitamin C along with electrolyte supplementation was found to ameliorate the HS in buffaloes ([Sunil Kumar et al., 2010](#)). Studies have shown that supplementation of vitamins C, E, and A and zinc is effective in preventing the negative effect of environmental stress. Acute administration of vitamin E (given at breeding; [Ealy, Aréchiga, Bray, Risco, & Hansen, 1994](#)) or β -carotene (given at d -6 to 0 before expected estrus; [Aréchiga et al., 1998](#)) did not improve fertility of cows inseminated during warm periods of the year in Florida. Long-term (≥ 90 d) feeding of supplemental β -carotene did increase herd-pregnancy rate for cows calving in Florida from May 2 to August 5 ([Aréchiga et al., 1998](#)). The proportion of cows pregnant by d120 postpartum was 21% in controls and 35% for cows fed supplemental β -carotene.

- Addition of feed additives/vitamins and mineral supplementations that helps in increasing feed intake, modify gut microbial population and gut integrity and maintain proper cation and anion balance. Feeding diets that have a high dietary cation–anion difference (DCAD) improved DMI and milk yield (Tucker, Harrison, & Hemken, 1988). During HS conditions, DMI was improved as DCAD was increased from 12.0 to 46.4 meq Na + K – Cl/100 g feed DM, regardless of whether Na or K was used to increase DCAD (West, Haydon, Mullinix, & Sandifer, 1992).
- Cows in HS conditions are prone to rumen acidosis, which makes fiber quality especially important to maximize rumen buffering and saliva production. Feeding a high-quality bypass fat remains an effective way to provide an energy-dense diet at a time when cows are eating fewer pounds of feed.

Production Adjustments

Changes in livestock practices include (1) diversification, intensification and/or integration of pasture management, livestock and crop production; (2) changing land use and irrigation; (3) altering the timing of operations; and (4) conservation of nature and ecosystems; (5) introducing mixed livestock farming systems, such as stall-fed systems and pasture grazing.

CONCLUSION

Climate change has been recognized as the foremost environmental problem of the 21st century and has far-reaching consequences for dairy and meat production, especially in vulnerable parts of the world where it is vital for nutrition and livelihoods. The molecular and cellular mechanisms of heat-induced cell damage, especially the deleterious effect of heat on puberty, oocyte, or embryo viability are poorly understood. Recent studies on in vitro heat shock to oocytes and embryos have helped in resolving the mechanism of heat-induced changes in developmental potential or damage to bovine oocytes and embryos. Therefore, identification and characterization of cellular thermoprotective molecules can be considered an alternative to modulate the effects of elevated temperature in reproductive function.

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Chapter 9

Impact of Climate Change on Fisheries

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INTRODUCTION

Climate of a region indicates average weather for a longer period, whereas climate change represents a statistically significant change in either the mean state of the climate or its statistical properties, persisting for an extended period particularly decades or longer. Climate change is not only a major global environmental problem, but also an issue of great worry for the developing country like India. As to global climate change, it is the phenomenon where other climatic factors change. Oceans and seas are mostly affected by the process of change caused by global warming as they constitute a large portion of our planet and have rich biodiversity. A temperature increase of only a few degrees does not cause an increase in the temperature of large water masses such as oceans, seas, lakes, and ponds but it give rise to hydrological events that cause a change in the physical and chemical characteristics of water. We now are entering an era where global climate change, brought about by rising levels of greenhouse gases, has the potential to alter the distribution of life on the planet as we know it. But, what exactly is that new temperature? What will be the attendant consequences of that temperature rise on the diversity of fishes on planet? The environmental science community has worked feverishly for most of the last two decades to answer just that question (IPCC, 2007). The current expectation is that there will be at least doubling of the mid-1980s level CO₂ emissions by the year 2050. Climate change has been recognized as the foremost environmental problem of the 21st century and has been a subject of considerable debate and controversy. It is predicted to lead to adverse, irreversible impacts on the earth and the ecosystem as a whole. Although it is difficult to connect specific weather events to climate change, increases in global temperature have been predicted to cause broader changes, including glacial retreat, arctic shrinkage, and worldwide sea level rise. Climate change has been implicated in mass mortalities of several aquatic species including plants, fish, corals, and mammals.

EFFECT OF CLIMATE CHANGE

Climate change is the variation in the earth's global climate or in regional climates over time, and it involves changes in the variability or average state of the atmosphere over durations ranging from decades to millions of years. The United Nations Framework Convention on Climate Change (UNFCCC) uses the term "climate change" for human-caused change and "climate variability" for other changes. In last 100 years, ending in 2005, the average global air temperature near the earth's surface has been estimated to increase at the rate of $0.74^{\circ}\text{C} \pm 0.18^{\circ}\text{C}$ ($1.33^{\circ}\text{F} \pm 0.32^{\circ}\text{F}$) (IPCC, 2007). In recent usage, especially in the context of environmental policy, the term "climate change" often refers to changes in the modern climate (Fig. 9.1).

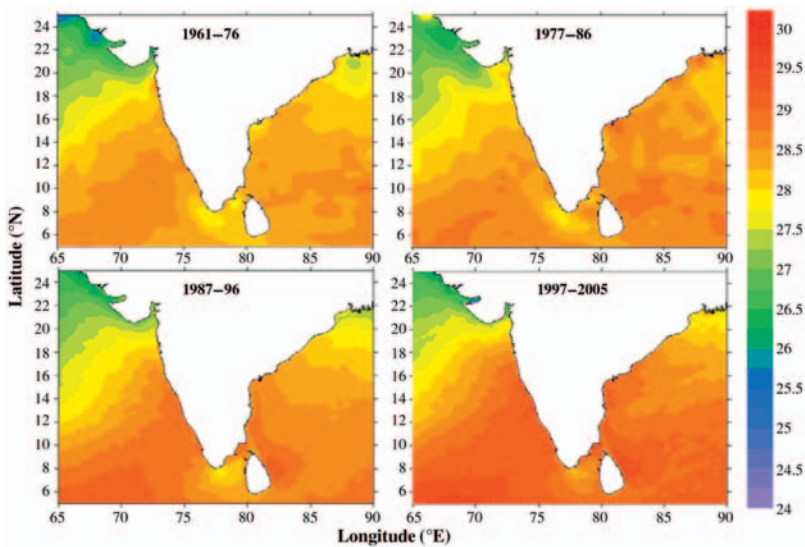


FIGURE 9.1 Rise in sea surface (°C) temperature in the Indian seas (CMFRI, 2009).

Global Warming

“Global warming is the process in which the earth’s temperature and the temperature on the atmosphere layers that are close to earth rise artificially as a result of the intense increase in some gases that occur in consequence of various human activities and that are qualified as greenhouse gases in the atmosphere.” As to global climate change, it is the phenomenon where other climatic factors change as well depending upon global warming. Oceans and seas are mostly affected by the process of change caused by global warming, as they constitute a large portion of our planet and have rich biodiversity. A temperature increase of only a few degrees does not only cause for increase in the temperature of large water masses such as oceans, seas, lakes, and ponds but also causes hydrological events that cause a change in the physical and chemical characteristics of water. Water temperature is the most important environmental parameter that affects the life cycle, physiology, and behaviors of aquatic living beings. Therefore, to what extent the oceans and seas will be affected by global warming on a worldwide scale, how global warming will affect the distribution of species, the relationship between global warming and biodiversity, and the impact of climate change on water resources which can renew themselves but are limited are topics that need to be considered carefully.

NATURAL PROCESSES AFFECTING THE EARTH’S TEMPERATURE

Sun is the primary source of energy on earth. Though the sun’s output is nearly constant, small changes over an extended period of time can lead to

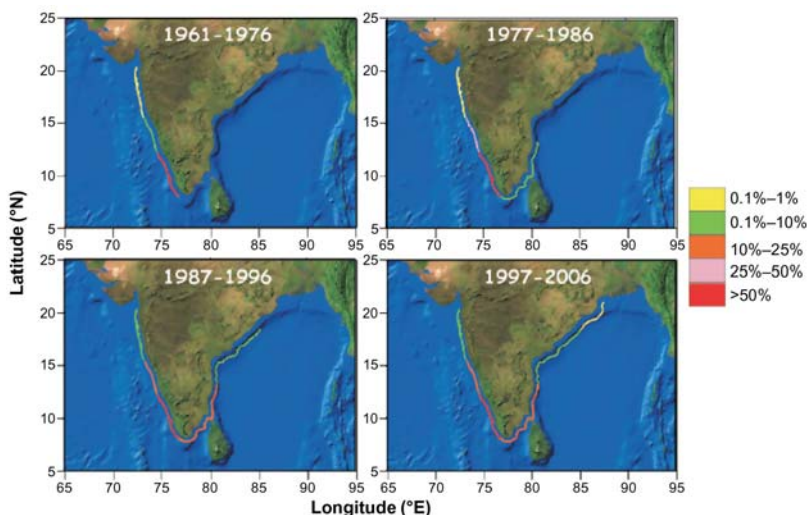


FIGURE 9.2 Extension in the boundaries of Sardine (Vivekanandan, Rajagopalan, & Pillai, 2009).

climate change. The earth's climate changes are in response to many natural processes like orbital forcing (variations in its orbit around the Sun), volcanic eruptions, and atmospheric greenhouse gas concentrations. Changes in atmospheric concentrations of greenhouse gases and aerosols, land cover and solar radiation alter the energy balance of the climate system and cause warming or cooling of the earth's atmosphere. Volcanic eruptions emit many gases and one of the most important of these is sulfur dioxide (SO_2) which forms sulfate aerosol (SO_4) in the atmosphere (Fig. 9.2).

Greenhouse Effect

Although the gases that are found in the atmosphere and called greenhouse gases directly permeate most of the short wave rays coming directly from the sun, they trap most of the long wave rays that are radiated back to the atmosphere after the warming of the earth. This feature of the atmosphere is called the "Natural Greenhouse Effect." A change that occurs in the rates of greenhouse gases in the atmosphere disturbs the present natural balance of the atmosphere. These result in an increase or decrease in temperature on a global basis. Greenhouse gases that cause greenhouse effect are carbon dioxide, chlorofluorocarbon gases, methane, nitrous oxides, ozone, and water vapor. Their characteristics, shares in global warming, and densities in the atmosphere are different from each other. As carbon dioxide largely permeates the short wave rays coming directly from the sun but traps the long wave rays radiated back from the earth, it is a greenhouse gas with a very important role in the warming of the lower parts of the atmosphere.

A percentage of 50–60 anthropogenic greenhouse effect is caused by this gas. According to the mathematical computer models developed by scientists lately, it has been calculated that in the event that the CO₂ density is doubled, the global temperature will rise by 3°C. This result gives an idea about how high the impact level of carbon dioxide on global warming is. Therefore, among the precautions to be taken against global warming, decreasing of the carbon dioxide emission comes first, and remarkable efforts are made for this purpose on an international level. Measurements taken in Hawaii as from 1958 have revealed that apart from its seasonal emissions, CO₂ increases year by year as well. Rising air temperatures affect the physical nature of our oceans. As air temperatures rise, water becomes less dense and separates from a nutrient-filled cold layer below. This is the basis for a chain effect that impacts all marine life who counts on these nutrients for survival.

Melting of Sea and Continent Glaciers

Glaciers are the second largest water depositories following the oceans and the largest freshwater depositories; they constitute 98.5% of freshwater. Glaciers are rapidly undergoing changes all around the world. In the 20th century, Mount Kilimanjaro in Africa lost approximately third-fourth of its glacial mass. The mass of the glaciers in Caucasus decreased by half and the glaciers on Tien Shan Mountains on the Chinese–Russian border shrank by 20% in the last 40 years. The glaciers in New Zealand lost one-fourth of their masses in 20 years. The number of glaciers in Spain which was 27 in 1980 is 13 today. Although the Qori Kalis glacier on Andes Mountains in Peru regressed 4 m per year between 1963 and 1978, this regression speed reached 30 m in 1995. Himalayan glaciers in Garhwal are melting at a great speed. Researchers believe that the glaciers in the central and western parts of Himalayas will have disappeared by 2035. Decreasing of snow due to climate changes in Southwestern Asia, particularly on Himalayas, leads to the reflection of less sun rays back to the space and increasing of the temperature on earth. The growing difference in temperature between the land and the sea strengthens the monsoon winds. And the monsoon winds that blow more strongly stir the seas and carry the nutrients to the surface and cause phytoplankton blooms. Satellite data indicate that since 1978, the average annual North Pole ice size have become 2.7% smaller for each 10 years, and this value is much higher in summers; it is 7.4% for each 10 years (Fig. 9.3).

Rise in the Sea Level

Ice melts in parallel with the increase in temperature, and thus, the amount of water that flows into the seas from the glaciers and ice caps increases. The waters of oceans become warmer and their volumes expand. Intergovernmental Panel on Climate Change (IPCC) states that in the last

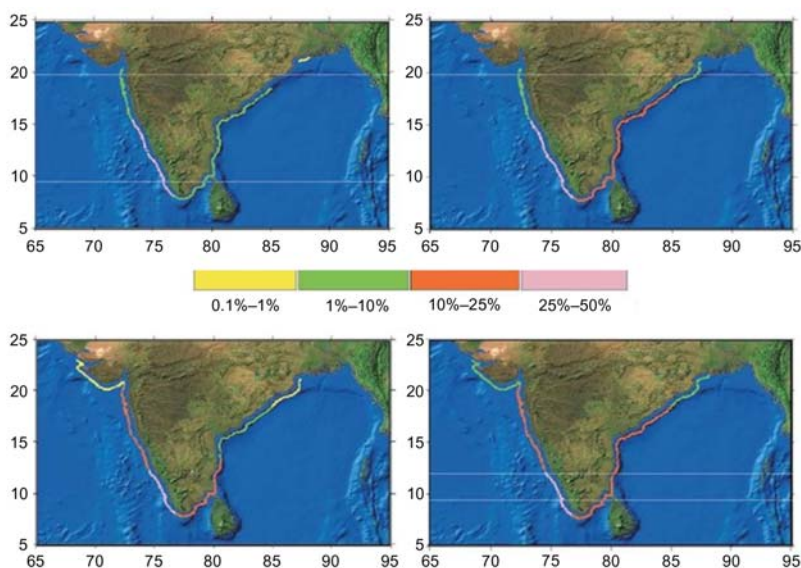


FIGURE 9.3 Extension in the boundary of Mackerel (CMFRI, 2011).

century, the sea level rose by 10–20 cm on a global scale, and this was mainly caused by global warming and that in this century, it will rise by another 40–60 cm. Depending on the rise in the sea level, erosion on the coast and the coastal habitat will be seen; and as a result, the problem of salinity in freshwater reserves, change in tidal amplitude in the gulfs, chemical and microbiological pollution on the coastlines, and an increase in coastal floods will occur.

Degradation in the Coastal Ecosystem

The main impact of global warming and climate change will be seen on coasts, the most productive areas of seas. Experts conducting studies on coasts have calculated that each rise of 1 cm in the sea level may cause a horizontal regression with a width of 1 m on sandy coastal lines due to erosion. The changes in the sea water level will endanger the coastal habitat and species, such as the sea turtle which uses the beaches as reproduction areas and lays eggs; they will be unfavorably affected, as their reproduction areas will become narrower.

Change in Currents

In the oceans, there are large currents that are active. These currents have remarkable impacts on water cycle and weather conditions. The differences

that occur in the sea water density depending on the salinity rate and temperature of the water, and the winds and storms cause currents. As a result, cold-water currents such as Humboldt and hot water currents such as Gulf Stream occur, and these affect the surrounding climate conditions. The researches have put forward that the glaciers in the North Pole will melt due to global warming and that the glaciers that will have melted may completely stop the Gulf Stream hot water current in 10 years. The complete discontinuation of the flow of the Gulf Stream may take the world to a point of no return. It may cause the northern hemisphere of the earth to begin a new glacial era, as Gulf Stream reaches Northern Europe from Mexican Gulf over the Atlantic Ocean and makes the climates of England, Ireland, northern parts of France, Belgium, the Netherlands, and Germany become temperate. Besides, the plankton carried through the ocean with the effect of Gulf Stream, that is, the first links of the food chain, will have disappeared and all the food links, mainly the fish that feed on these planktons, will suffer. Extinction of these living beings will result in the starvation of the living beings feeding on them, and this will affect the humans, an important link of the food chain.

Increase in the Distribution Areas of Vectors

As a result of the warming of sea water, the number of bacteria living in high temperature and their disease causing capacities will increase more. For this reason, global warming poses danger for fish breeding performed in seas. What is known is that most coral diseases occur at higher-than-normal sea-water temperatures. As temperatures are expected to rise considerably during the next few decades, it is likely that coral diseases will become more prevalent. The models predict that in the event of an increase by 3–5 degrees in the earth's temperature until 2100, malaria prevalence will affect 45%–60% of the world's population until the second half of the next century. This implies a patient increase of 50–80 million per year. An increase in diseases such as salmonellosis and cholera is also expected as a result of high temperature and floods (Fig. 9.4).

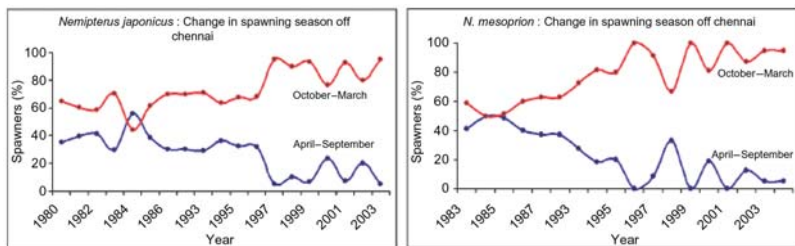


FIGURE 9.4 Change in spawning season of *Nemipterus japonicus* and *N. mesoprius* off Chennai (Vivekanandan and Rajagopalan, 2009).

Increase in the Extinction of Species

As the breaking of the nourishment chain in nature once will lead to incredible results, the extinction of some species will directly affect the other species. In the 2007 report of Intergovernmental Panel on Climate Change, it is stated that if the average temperature of the earth increases by 1–2°C, one-third of the species on the earth will change their distribution areas or become extinct.

IMPACT OF GLOBAL WARMING ON AQUATIC ORGANISMS

Plankton

Along with global warming, precipitation regime will change due to changing atmospherical rhythm, nourishing loads will suddenly enter the sea, and thus, seasonal plankton blooms will be probable. As the case is today, with the accumulation of organic materials that reproduce more than they are consumed on the bottom and their degrading the marine sulfates to sulfurs, living beings' life will be trapped in a narrow zone. Fish such as anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) feed on plankton and make the absorption of organic loads in the water colony possible. In a process where this does not happen, that is, where the plankton drift in the bottom, H₂S formation in the bottom will speed up and thus, with the decreasing of fish consuming organic materials in the system, H₂S layer will rise even more.

Crustaceans

The increase in the CO₂ amount which is among the causes of climate change results in the decline of sea water's pH, and thus the acidification of the sea water. This case negatively affects the crustaceans. The outer skeletons of crustaceans consist of aragonite, a common form of calcium carbonate, and it can dissolve in acidic sea water. Extinction of these small crustaceans found at the bottom of the nutrition chain can change the entire sea ecosystem. Another negative impact that climate change has caused on crustaceans comes out with the regression of sea ice. Oceanographers point out that small crustaceans that are called krill and that feed on phytoplankton have decreased by 80% on average in the past 30 years. They attribute this case to the decreasing of the ice cover carried by South Ocean in winter, as winter ice is of great importance for the life cycle of algae and krill feed on algae. Extinction of krill that are the nutrition resource for many sea mammals and living beings in the sea may mean the destruction of the food chain in the South Ocean.

Coral Reefs

Coral reefs are very important for oceans as these reefs are the places where the carbon cycle takes place. Coral reefs constitute a natural set against big

ocean waves and tidal waves and serve for the protection of coastal lines. The fish and other crustaceans they hold are a significant source of food and mainstay. Reefs are places where many large living beings come to reproduce. Fish that are densely caught by humans either reproduce at these reefs or use the food produced by these reefs. If we consider that these form a chain in the shape of a pyramid, the destruction of the order in the reefs means the destruction of this chain. Tropical coral reefs are increasingly threatened by shifts in the world's climate, overfishing and declining water quality. High levels of genetic diversity within populations of corals are likely to be an important element in evolutionary responses to climate change. Populations at high latitudes may be more vulnerable to climate change because they are typically at the margins of geographical ranges and are likely to be small and isolated. Corals grow as long as they are not broken because of external influences. However, the changes in the temperature and acidity rates kill and solidify the corals. The dead corals that are white-gray in color cannot grow any more. For instance, in Caribbean in 1989–90, the increase in the temperature of the sea water by 2°C, that is, rising of the water temperature to 30–31°C from 28–29°C, has caused mass death of corals. In fact, extinction of corals does not only lead to the destruction of biodiversity in the seas, but also cause a decrease in the absorption of CO₂, which is responsible for global warming at the first rank, by the seas. Such processes are interpreted as the sign of the global collapse of the system by the experts.

Fish

Water temperature comes first in the list of most determinant factors as it is essential for the reproduction of fish species and the formation of an ideal living environment. In preadolescence stages called larva and juvenile, fish are quite susceptible to changes in the water temperature. A fish population can be tolerant of temperature changes in the area where it is distributed in a certain time interval. If these changes are within a certain temperature boundary and slow, it generally causes migration of fish. Temperature takes important physiological phenomena such as feeding, respiration, osmoregulation, growth, and reproduction under control. If the individuals of population cannot adjust themselves according to the sudden and strong changes in temperature, one or some of their metabolism activities may deteriorate and mass deaths may occur. If long-term temperature increases are observed in a region, in any stock that is prevalent in that area, shifting of the southern border of the ovulation area toward the north, changing of ovulation areas horizontally with any increase in the bottom water temperature, preference of northern latitudes as new areas of feeding and growth, increase in food salts and amounts with the increase in temperature on higher latitudes and

changes in the present currents, prolongation of the growth period in a year, and shifting of the limit within which larvae may live toward northern latitudes may be observed. For instance, that the fish of *Thallossoma pavo* species can now be observed in the Sea of Marmara and that its distribution has shifted toward the north from the south of the Mediterranean Sea is explained with the impacts of global warming. The number of Indian Ocean-origin fish found within the waters of our country is more than 30 already. One of the main reasons for the entrance of all these species into eastern Mediterranean and their competition for areas with local species is the increase in water temperature in the Mediterranean. The hot lower current with high salinity from the Mediterranean to the Black Sea and the cold upper current with low salinity from the Black Sea regulate the distribution and migration of living beings in the sea. The increase in the sea water temperature affects the transition and entrance of thermophilic fish species into the Black Sea. Fish such as *Sardinella aurita*, *Boops boops*, and *Sarpa salpa* living in the Mediterranean were very rarely seen in the Black Sea and the Marmara 20 years ago. Today, they are frequently observed in those seas and even caught in the Western Black Sea, and this is attributed to the increase in the sea water temperature. Another effect of climate change is the decrease in the pH level of the sea water through increasing atmospheric CO₂.

There are two general physical effects of ocean warming on marine populations that are crucial to consider:

- Changes in natural habitats and food supply
- Changing ocean chemistry/acidification

CHANGES IN NATURAL HABITATS AND FOOD SUPPLY

Photosynthesis

Phytoplankton, one-celled plants that live at the ocean surface and algae use photosynthesis for nutrient fulfillment. Photosynthesis is a process that removes carbon dioxide from the atmosphere and converts it into organic carbon and oxygen that feeds almost every ecosystem. According to a recent NASA study, phytoplankton is more likely to thrive in cooler oceans. Similarly, algae, a plant that produces food for other marine life through photosynthesis, is vanishing due to ocean warming. As oceans are warmer, nutrients are blocked from traveling upward to these suppliers that are limited to a small surface layer and therefore cannot supplement marine life with necessary organic carbon and oxygen (Fig. 9.5).

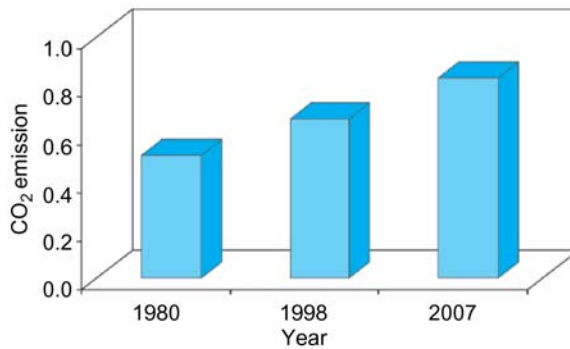


FIGURE 9.5 CO₂ emission ratio (tonnes of CO₂ per tonne of fish catch) by marine fishing boats in India during 1980, 1998, and 2007 (CMFRI, 2011).

Yearly Growth Cycles

Various plants and animals in our oceans need both a temperature and light balance in order to thrive. Temperature-driven creatures, such as phytoplankton, have started their yearly growth cycle earlier in the season due to warming oceans. Light-driven creatures start their yearly growth cycle around the same time. As phytoplankton is thriving in earlier seasons, the entire food chain is affected. Animals that once traveled to the surface for food are now finding an area void of nutrients, and light-driven creatures are starting their growth cycles at different times. This creates a nonsynchronous natural environment.

Migration

The warming of oceans may also lead to migration of organisms along the east and west coasts. Heat-tolerant species, such as shrimp, will expand northward, whereas heat-intolerant species, such as clams and flounder, will retreat northward. This migration will lead to a new mix of organisms in an entirely new environment, ultimately causing changes in predatory habits. If some organisms cannot adapt to their new marine environment, they will not flourish and die off.

CHANGING OCEAN CHEMISTRY/ACIDIFICATION

General Acidification

As carbon dioxide is being released into the ocean, the ocean chemistry drastically changes. Greater CO₂ concentrations released into our oceans create

increased ocean acidity. As ocean acidity increases, phytoplankton is reduced. This results in less ocean plants able to uptake greenhouse gases. In addition, increased ocean acidity threatens marine life, such as corals and shellfish, which may become extinct later this century from the chemical effects of CO₂.

Acidification Effect on Coral Reefs

Coral, one of the leading sources for the ocean's food and livelihood, is also changing with the onset of global warming. Naturally, coral secretes tiny shells of calcium carbonate in order to form its skeleton. Yet, as CO₂ from global warming is released into the atmosphere, acidification increases and the carbonate ions vanish. This results in lower extension rates or weaker skeletons in most corals.

Coral Bleaching

Coral bleaching, the breakdown in the symbiotic relationship between coral and algae is also occurring with warmer ocean temperatures. As Zooxanthellae, or algae, give coral its particular coloration, increased CO₂ in our oceans causes coral stress and a release of this algae. This leads to a lighter appearance. When this relationship that is so important for our ecosystem to survive vanishes, corals begin to weaken. Consequently, food and habitats for a great number of marine lives are also destroyed.

Holocene Climatic Optimum

This drastic climate change and its effect on surrounding wildlife are not new to us. The Holocene Climatic Optimum, a general warming period displayed in our fossil record from 9000 to 5000 B.P., proves that climate change can directly impact nature's inhabitants. In 10,500 B.P., younger dryas, a plant that was once spread throughout the world in various cold climates, became near extinct due to this warming period. Toward the end of the warming period, this plant that so much of nature had depended on was only to be found in the few areas that remained cold. Just as younger dryas became scarce in the past, phytoplankton, coral reefs, and the marine life that depend on them are becoming scarce in the present. Our environment is continuing on a circular path that may soon lead to chaos within a once naturally balanced environment.

Future Outlook and Human Effects

The warming of our oceans and its effect on marine life has a direct impact on us. As coral reefs die, we will lose an entire ecological habitat of fish.

According to the World Wildlife Fund, a small increase of 2°C would destroy almost all existing coral reefs. In addition, ocean circulation changes due to warming would have disastrous impacts on marine fisheries.

This drastic impact is often hard to imagine. It can only be related to a similar historical event. Fifty-five million years ago, ocean acidification led to a mass extinction of ocean creatures. According to our fossil record, it took more than 100,000 years for the oceans to recover. Eliminating the use of greenhouse gases and protecting our oceans will prevent this from reoccurring.

Rupture of the Food Chain

Another consequence of climate change, global warming, and the high absorption of CO₂ is the disruption of the marine food web. As mentioned earlier, absorption in large amounts of carbon dioxide would cause the disappearance of phytoplankton. Marine plant-feeding the small organisms such as krill, crustaceans, which in turn serve as food for marine species and such the blue whale and the humpback whale.

Other Species Affected

Other marine species such as penguins quickly lose their feeding areas and their natural habitat. Indeed, many penguin populations are already moving. According to the World Wildlife Fund (WWF) in 2007, there was a decline among Adelie penguins, whose total population was reduced by 65%, emperor penguins (50%), penguins Jugular (by 30%), and gentoo (60%). In addition, Orcas on the west coast of Canada are being affected in their diet. Apparently, the temperature rise in the Pacific Ocean has resulted in salmon with a drop of fat, forcing the whales to consume between 1.5 and 2 times more salmon than usual, sparking a growing obesity endangering the health of these animals. Similarly, in the Baltic Sea, thousands of ringed seal pups died in the 2008 to jump into the water without even enough fat for protection, or be prepared to feed themselves, particularly as a result of the thaw early in their caves. As per Chris Thomas, a scientist at the University of New York, from 10% to 50% of marine life will disappear in not much time, being the largest mass extinction that will be experienced from the time of the dinosaurs.

IMPACT OF CLIMATE CHANGE ON MARINE FISHERIES IN INDIA

Production from marine capture fisheries has been stagnant during the past 10 years because of overfishing, unregulated fishing, habitat destruction and pollution; climate change may exacerbate this situation. Warming of water may impact fish diversity, distribution, abundance, and phenology.

Acidification of water will affect calciferous animals. Storms, floods and drought will severely impair fisheries. Sea level rise will lower fish production and damage the livelihoods of communities. Some tropical fish stocks may face regional extinction. Some others may move toward higher latitudes. Coastal habitats and resources are likely to be impacted through sea level rise, warming sea temperatures, extremes of nutrient enrichment (eutrophication) and invasive species. Most fish species have a narrow range of optimum temperatures related to their basic metabolism and availability of food organisms. Even a difference of 10°C in sea water may affect their distribution and life processes. At shorter timescales of a few years, increasing temperature may result in changes in distribution, recruitment, and abundance. Species with short-life span and rapid turnover such as plankton and small pelagic fishes are most likely to experience such changes. At intermediate timescales of a few years to a decade, changes in distribution, recruitment, and abundance of many species may be acute. Changes in abundance will alter the species composition. At longer timescales of multidecades, changes in net primary production and transfer to higher trophic levels are possible.

Investigations carried out by the Indian Council of Agricultural Research show that different Indian marine species will respond to climate change as follows:

- Changes in species composition of phytoplankton may occur at higher temperature;
- Small pelagics may extend their boundaries;
- Some species may be found in deeper waters as well; and
- Phenological changes may occur.

Changes in Species Composition of Phytoplankton

Laboratory experiments on seven species of phytoplankton showed that some species may multiply faster at higher temperature (29°C) than at lower temperature (24°C). But they decay earlier at the higher temperature.

Small Pelagics Extend Their Boundaries

The oil sardine *Sardinella longiceps* and the Indian mackerel *Rastrelliger kanagurta* accounted for 21% of the marine fish catch in 2006. These small pelagics, especially the oil sardine, have been known for restricted distribution—between latitude 8°N and 14°N and longitude 75°E and 77°E (Malabar upwelling zone along the southwest coast of India) where the annual average SST ranges from 27°C to 29°C. Until 1985, almost the entire catch was from the Malabar upwelling zone; there was little or no catch from latitudes north of 14°N. During the last two decades, however, catches from latitude

14–20°N are increasing. In 2006, catches in this area accounted for about 15% of the all-India oil sardine catch. The higher the SST, the better the oil sardine catch. The surface waters of the Indian seas are warming by 0.04°C per decade. As the waters in latitudes north of 14°N are warming, the oil sardine and Indian mackerel are moving to northern latitudes. It is seen that catches from the Malabar upwelling zone have not gone down. Inference: The sardines are extending northward, not shifting northward. The Indian mackerel is also found to be extending northward in a similar way. According to CMFRI, the catch of oil sardines along the coast of Tamil Nadu has gone up dramatically, with a record landing of 185–877 t in 2006. The presence of the species in new areas is a bonus for coastal fishing communities. Assessing their socioeconomic needs will greatly help in developing coping strategies for adaptation to climate impacts. WWF is currently documenting community perceptions and experiences in relation to the oil sardine fishery of the eastern coasts.

Indian Mackerel Is Getting Deeper

Besides exploring northern waters, the Indian mackerel *R. kanagurta* has been descending deeper as well during the last two decades [CMFRI \(2008\)](#). The fish normally occupies surface and subsurface waters. During 1985–89, only 2% of the mackerel catch was from bottom trawlers, the remainder was caught by pelagic gear such as drift gillnet. During 2003–07, however, an estimated 15% of the mackerel has been caught by bottom trawlers along the Indian coast. It appears that with the warming of subsurface waters, the mackerel has been extending deeper and downward as well.

Spawning: Threadfin Breams Like It Cool

Fish have strong temperature preferences so far as spawning goes. The timing of spawning, an annually occurring event, is an important indicator of climate change. Shifts in the spawning season of fish are now evident in the Indian seas. The threadfin breams *Nemipterus japonicus* and *N. mesoprion* are distributed along the entire Indian coast at depths ranging from 10 to 100 m. They are short-lived (longevity: about 3 years), fast growing, highly fecund, and medium-sized fishes (maximum length: 35 cm). Data on the number of female spawners collected every month off Chennai from 1981 to 2004 indicated wide monthly fluctuations. However, a shift in the spawning season from warmer to relatively cooler months (from April–September to October–March) was discernible. Although 35.3% of the spawners of *N. japonicus* occurred in warm months during 1981–85, only 5.0% of the spawners occurred in the same season during 2000–04. What about the cool months? During 1981–85, 64.7% of the spawners occurred during October–March, whereas as high as 95.0% of the spawners occurred during

the same season in 2000–04. A similar trend was observed in *N. mesoprion* too. The occurrence of spawners of the two species decreased with increasing temperature during April–September, but increased with increasing temperature during October–March over the timescale. It appears that SST between 28°C and 29°C may be the optimum. When the SST exceeds 29°C, the fish shifts the spawning activity to seasons when the temperature is around the preferred optima. These changes may have an impact on the nature and value of fisheries (Perry, Low, Ellis, & Reynolds, 2005). If small-sized, low value fish species with rapid turnover of generations are able to cope up with changing climate, they may replace large-sized high value species, which are already declining due to fishing and other nonclimatic factors. Such distributional changes might lead to novel mixes of organisms in a region, leaving species to adjust to new prey, predators, parasites, diseases, and competitors and result in considerable changes in ecosystem structure and function.

False Trevally Populations Decline in the Gulf of Mannar

As part of WWF India-commissioned project, the Suganthi Devadason Marine Research Institute (SDMRI), Tuticorin, undertook a study in 2004 in the Gulf of Mannar region to analyze the effect of climate change on the fishery of False Trevally (*Lactarius lactarius*) and the reduction in the income of small-scale fishermen. The project helped identify the migratory patterns of the fish species. False Trevally is an economically and culturally important fish in India and found near the Rameshwaram coast of south east India. The species is generally seen at depths ranging from 15 to 90 m. But over the past few years, there has been a steady decline in the catch of this fish—both because of human disturbance and changes in ocean temperatures. Destructive fishing practices have also led to decline of the species. Result: the species has moved to other regions along the coast including the east coast of Sri Lanka. Currently, it is difficult to find out how much of catch fluctuation is due to changes in fish distribution. A time series analysis on stock biomass of different species along the Indian coasts does not exist. Moreover, catches are influenced by economic factors such as the relative price paid for different types of fish, and changes in fishing methods or fishing effort. For instance, introduction of mechanized craft in the 1960s, motorized craft in the 1980s, and large trawlers for multiday fishing in the 1990s substantially increased the fish catch along the Indian coast. These nonclimatic factors often obscure climate-related trends in fish abundance. Perhaps a detrending analysis for removing the impact of nonclimatic factors may help arrive at conclusions on the impact of climate change on marine fisheries. The effects of changed fish migration and distribution caused by climate change are most difficult to deal with for highly migratory species, such as tuna. It is not clear whether the spurt in yellowfin tuna fishery in the

Bay of Bengal and eastern Arabian Sea in the last five years is due to climate-driven changes in the migration route of the fish.

Footprint of Fishing Operations

Fisheries activities contribute to GHG emissions during capture operations and subsequently during the transport, processing, and storage of product. Industrial fisheries have much greater emissions than small-scale fisheries. The estimated fuel consumption by global capture fisheries operations ranges from 14 to 42 million tonnes equivalent to CO₂ emission of 43 to 134 Tg [1 Tg (teragram) = 1 million tonnes] (Tyedmers, Watson, & Pauly, 2005; FAO, 2007). It is estimated that global fisheries account for about 1.2% of the global oil consumption.

There are 58,911 mechanized and 75,591 motorized marine fishing boats in India. All these boats use diesel for propulsion, and among these, trawlers use diesel for propulsion as well as fishing. By undertaking survey on diesel consumption by 1332 mechanized boats and 631 motorized boats operating from major fishing harbors in Kerala, Tamil Nadu, Maharashtra, and Gujarat, CMFRI (2009) has estimated that annual fossil fuel consumption by marine fishing boats in India is around 912 million liters per year, which is equivalent to CO₂ emission of 2.4 million tonnes per year during 2005–07. CO emission is generally considered as 10% of CO₂ emission, which is equivalent to 0.24 million tonnes per year.

Coral Reefs May Become Remnants

Coral reefs are the most diverse marine habitat, which support an estimated 1 million species globally. They are highly sensitive to climatic influences and are among the most sensitive of all ecosystems to temperature changes, exhibiting the phenomenon known as coral bleaching when stressed by higher than normal sea temperatures. Corals usually recover from bleaching, but die in extreme cases. In the Indian seas, coral reefs are found in the Gulf of Mannar, Gulf of Kutch, Palk Bay, Andaman Sea, and Lakshadweep Sea. Indian coral reefs have experienced 29 widespread bleaching events since 1989, and intense bleaching occurred in 1998 and 2002 when the SST was higher than the usual summer maxima. By using the relationship between past temperatures and bleaching events and the predicted SST for another 100 years, projected the vulnerability of corals in the Indian Seas. They believe that the coral cover of reefs may soon start declining. The number of decadal low-bleaching events will remain between 0 and 3 during 2000–89, but the number of decadal catastrophic events will increase from 0 during 2000–09 to 8 during 2080–89. Given the implication that reefs will not be able to sustain catastrophic events more than three times a decade, reef-building corals are likely to disappear as dominant organisms on coral reefs

between 2020 and 2040. Reefs are likely to become remnant between 2030 and 2040 in the Lakshadweep Sea and between 2050 and 2060 in other regions in the Indian seas. These projections take into consideration only the warming of sea water. Other factors such as increasing acidity of sea water are not considered. If acidification continues in future as it does now, all coral reefs would be dead within 50 years. Given their central importance in the marine ecosystem, the loss of coral reefs is likely to have several ramifications.

Impacts of Climate Change on Coastal Systems

Coastal India (with over 8000 km of coastline) is a productive and ecologically diverse landscape. Climate change may aggravate the impact of injurious large-scale development and reduce the productivity of marine ecosystems.

The Fourth Assessment Report of the [IPCC \(2007\)](#) has suggested that climate change is likely to significantly impact coastal India. Some possible impacts are as follows:

- More hot days, more heat waves, more death from heat strokes in recent years;
- Intrusion of saline water into groundwater in coastal aquifer; and
- Decline in precipitation, droughts in most delta regions of India, and drying of wetlands.

Worldwide, WWF studies have brought out some important underlying impacts of climate change on marine ecosystems—such as a rise in SST, decreasing marine pH, shifting ocean currents, release of methane hydrates, and rising sea level. India is vulnerable to major climate changes because of a long coastline on the east and west and the Himalayan mountain range in the north. WWF-India has been working in some of India's most critical ecosystems and landscape. Its studies seek to probe climate impacts in the Sundarbans, the coastal regions of south India and in the Himalayas, and focus mainly on impacts and adaptation; mitigation; and policy interventions.

Sundarbans

The Sundarbans is part of the world's largest delta (80,000 sq. km) formed from sediments deposited by three great rivers—the Ganges, Brahmaputra, and Meghna. It consists of 102 low lying islands in the Bay of Bengal and forms one of the world's richest mangrove ecosystems (34 mangrove species). Faunal diversity is significant too, with a strong tiger population. The combination of terrestrial, freshwater, and marine flora and fauna makes this one of the most diverse and productive ecosystems in the country. The Sundarbans is now under severe stress due to sea level rise and associated

problems. A population of 4 million in the Indian Sundarbans is severely stressed. Mangroves are under threat, so are endangered species like tigers and turtles. An effective coping mechanism to reduce the vulnerability of the region is essential. WWF-India is documenting local community knowledge and climate perceptions through an initiative known as “Climate Witness.” It was launched because of the strong indicators of climate change from various scientific studies. WWF India hopes that this initiative will make the authorities integrate climate change concerns into development planning through a bottom-up approach.

Characteristics of the initiative:

- Stakeholders at different levels will help develop a model intervention;
- A homogeneous geographical area will be identified to validate the model; and
- Local concerns on climate change will be integrated into development planning.

Islands selected for the “Climate Witness” initiative studies are mostly in the southwestern corner of Sundarbans (except Chhoto, Mollakhali, and Bali islands situated in the northeastern part of the delta). Local communities in these sites were more concerned by weather phenomena (such as monsoon delays in recent years), than by rising temperatures. Local residents reported very high frequency of thunder and lightning during storms in last 10–15 years. In their opinion, depression and cyclonic storms occurred more frequently than earlier. Delayed monsoons and untimely rain-impaired agricultural productivity leading to loss of crops and increased pest attacks.

Climate Change Impacts on Inland Fisheries—The Indian Scenario

In recent years, the climate is showing perceptible changes in the Indian sub-continent, where the average temperature is on the rise over the last few decades. In India, observed changes include an increase in air temperature, regional monsoon variation, frequent droughts, and regional increase in severe storm incidences in coastal states and Himalayan glacier recession (Vass, Das, Srivastava, & Dey, 2009). In some states like West Bengal, the average minimum and maximum temperatures have increased in the range of 0.1–0.9°C throughout the state. The average rainfall has decreased and monsoon is also delayed; consequently, the climate change impact is being felt on the temperature of the inland water bodies and on the breeding behavior of fishes. It is well known that temperature is an important factor which strongly influence the reproductive cycle in fishes. Temperature, along with rainfall and photoperiod, stimulate the endocrine glands of fishes which help in the maturation of the gonads. In India, the inland aquaculture is centered on the Indian major carps, *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*,

and their spawning occurs during the monsoon (June–July) and extends till September. In recent years, the phenomenon of IMC maturing and spawning as early as March is observed, making it possible to breed them twice a year. Thus, there is an extended breeding activity as compared to a couple of decades ago (Dey et al., 2007), which appears to be a positive impact of the climate change regime. The mighty river Ganga forms the largest river system in India and not only millions of people depend on its water but it provides livelihood to a large group of fishermen also. The entire length of the river, with a span of 2525 km from source to mouth is divided into three main stretches consisting of upper (Tehri to Kanauji), middle (Kanpur to Patna), and lower (Sultanpur to Katwa). From analysis of 30 years' time series data on river Ganga and water bodies in the plains, Vass et al. (2009) reported an increase in annual mean minimum water temperature in the upper cold-water stretch of the river (Haridwar) by 1.5°C (from 13°C during 1970–86 to 14.5°C during 1987–2003) and by 0.2–1.6°C in the aquaculture farms in the lower stretches in the Gangetic plains. This change in temperature climate has resulted in a perceptible biogeographically distribution of the Gangetic fish fauna. A number of fish species which were never reported in the upper stretch of the river and were predominantly available in the lower and middle stretches in the 1950s have now been recorded from the upper cold-water region. Among them, *Mastocembelus armatus* has been reported to be available at Tehri-Rishikesh, and *Glossogobius gurius* is available in the Haridwar stretch (Sinha, De, & Jha, 1998), and *Xenentodon cancila* has also been reported in the cold-water stretch (Vass et al., 2009). The predator–prey ratio in the middle stretch of the river has been reported to be declined from 1:4.2 to 1:1.4 in the last three decades. Fish production has been shown to have a distinct change in the last two decades where the contribution from IMCs has decreased from 41.4% to 8.3% and that from catfishes and miscellaneous species increased (Vass et al., 2009).

Anticipated Impacts in Next Few Decades

In addition to incremental changes of existing trends, complex social and ecological systems, such as coastal zones and fisheries, may exhibit sudden qualitative shifts in behavior when forcing variables past certain thresholds (Daw, Adger, Brown, & Badjeck, 2009). For example, IPCC originally estimated that the Greenland ice sheet would take more than 1000 years to melt, but recent observations suggest that the process is already happening faster owing to mechanisms for ice collapse that were not incorporated into the projections (Lenton et al., 2008). The infamous collapse of the Northwest Atlantic northern cod fishery provides a nonclimate-related example where chronic overfishing led to a sudden, unexpected, and irreversible loss in production from this fishery. Thus, existing observations of linear trends cannot be used to reliably predict impacts within the next 50 years (Daw et al., 2009).

A study by [Veron et al. \(2009\)](#) also emphasizes impact of increasing atmospheric CO₂ levels due to global warming on mass coral bleaching worldwide. According to this group, temperature-induced mass coral bleaching causing mortality on a wide geographic scale started when atmospheric CO₂ levels exceeded approximately 320 ppm. At today's level of approximately 387 ppm, allowing a lag-time of 10 years for sea temperatures to respond, most reefs worldwide is committed to an irreversible decline. Mass bleaching will in future become annual, departing from the 4 to 7 years return time of El Niño events. Bleaching will be exacerbated by the effects of degraded water quality and increased severe weather events. In addition, the progressive onset of ocean acidification will cause reduction of coral growth and retardation of the growth of high magnesium calcite-secreting coralline algae. If CO₂ levels are allowed to reach 450 ppm (due to occur by 2030–40 at the current rates), reefs will be in rapid and terminal decline worldwide from multiple synergies arising from mass bleaching, ocean acidification, and other environmental impacts. Damage to shallow reef communities will become extensive with consequent reduction of biodiversity followed by extinctions. Reefs will cease to be large-scale nursery grounds for fish and will cease to have most of their current value to humanity. There will be knock-on effects to ecosystems associated with reefs, and to other pelagic and benthic ecosystems. This is likely to have been the path of great mass extinctions of the past, adding to the case that anthropogenic CO₂ emissions could trigger the Earth's sixth mass extinction ([Veron et al., 2009](#)).

Adaptation and Mitigation Options

Adaptation to climate change is defined in the climate change literature as an adjustment in ecological, social, or economic systems, in response to observed or expected changes in climatic stimuli and their effects and impacts in order to alleviate adverse impacts of change, or take advantage of new opportunities. Adaptation is an active set of strategies and actions taken by peoples in response to, or in anticipation to the change in order to enhance or maintain their well-being. Hence, adaptation is a continuous stream of activities, actions, decisions, and attitudes that informs decisions about all aspects of life and that reflects existing social norms and processes ([Daw et al., 2009](#)).

Many capture fisheries and their supporting ecosystems have been poorly managed, and the economic losses due to overfishing, pollution, and habitat loss are estimated to exceed \$50 billion per year ([World Bank & FAO, 2008](#)). The capacity to adapt to climate change is determined partly by material resources and also by networks, technologies, and appropriate governance structures. Improved governance, innovative technologies, and more responsible practices can generate increased and sustainable benefits from fisheries. There is a wide range of potential adaptation options for fisheries. To build

resilience to the effects of climate change and derive sustainable benefits, fisheries and aquaculture managers need to adopt and adhere to best practices such as those described in the FAO “Code of Conduct for Responsible Fisheries,” reducing overfishing and rebuilding fish stocks. These practices need to be integrated more effectively with the management of river basins, watersheds, and coastal zones. Fisheries and aquaculture need to be blended into National Climate Change Adaptation Strategies. In absence of careful planning, aquatic ecosystems, fisheries, and aquaculture can potentially suffer as a result of adaptation measures applied by other sectors such as increased use of dams and hydropower in catchments with high rainfall, or the construction of artificial coastal defenses or marine wind farms.

Mitigation solutions reducing the carbon footprint of fisheries and aquaculture will require innovative approaches. One example is the recent inclusion of mangrove conservation as eligible for reducing emissions from deforestation and forest degradation in the developing countries, which demonstrates the potential for catchment forest protection. Other approaches to explore include finding innovative but environmentally safe ways to sequester carbon in aquatic ecosystems, and developing low-carbon aquaculture production systems.

There is mounting interest in exploiting the importance of herbivorous fishes as a tool to help ecosystems recover from climate change impacts. Aquaculture of herbivorous species can provide nutritious food with a small carbon footprint. This approach might be particularly suitable for recovery of coral reefs, which are acutely threatened by climate change. Surveys of 10 sites inside and outside a Bahamian marine reserve over a 2.5-year period demonstrated that increases in coral cover, including adjustments for the initial size distribution of corals, were significantly higher at reserve sites than those in nonreserve sites: macroalgal cover was significantly negatively correlated with the change in total coral cover over time. Reducing herbivore exploitation as part of an ecosystem-based management strategy for coral reefs appears to be justified (Mumby & Harborne, 2010). Furthermore, farming of shellfish, such as oysters and mussels, is not only good business, but also helps clean coastal water, whereas culturing aquatic plants help to remove waste from polluted water. In contrast to the potential declines in agricultural yields in many areas of the world, climate change opens new opportunities for aquaculture as increasing numbers of species are cultured. Marine fish is one of the most important sources of animal protein for human use, especially in the developing countries with coastlines. Marine fishery is also an important industry in many countries. The depletion of fishery resources is happening mainly due to anthropogenic factors such as overfishing, habitat destruction, pollution, invasive species introduction, and climate change. The most effective ways to reverse this downward trend and restore fishery resources are to promote fishery conservation, establish marine protected areas, adopt ecosystem-based management, and implement a “precautionary principle.” In addition,

enhancing public awareness of marine conservation, which includes ecolabeling, fishery ban or enclosure, slow fishing, and MPA (marine protected areas) enforcement is important and effective (Shao, 2009).

The assessment report of the fourth International Panel on Climate Change confirms that global warming is strongly affecting biological systems and that 20%–30% of species risk extinction from projected future increases in temperature. One of the widespread management strategies taken to conserve individual species and their constituent populations against climate-mediated declines has been the release of captive-bred animals to wild in order to augment wild populations for many species. Using a regression model based on a 37-year study of wild and sea-ranched Atlantic salmon (*Salmo salar*) spawning together in the wild, McGinnity et al. (2009) showed that the escape of captive-bred animals into the wild can substantially depress recruitment and more specifically disrupt the capacity of natural populations to adapt to higher winter water temperatures associated with climate variability, thus increasing the risk of extinction for the studied population within 20 generations. According to them, positive outcomes to climate change are possible if captive-bred animals are prevented from breeding in the wild. Rather than imposing an additional genetic load on wild populations by releasing maladapted captive-bred animals, they propose that conservation efforts should focus on optimizing conditions for adaptation to occur by reducing exploitation and protecting critical habitats.

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Chapter 10

Nanotechnology for Sustainable Agriculture

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NANOTECHNOLOGY: DEFINITION

The term nanotechnology is commonly defined as the science and technology attributed to the application, usage, and manipulation of material and matter at a scale one-billionth of a meter (Regis, 1995). The term, meaning “small” or “dwarf” in Latin, has dwarfed most established technologies by its sheer size and scale. The definition is intriguing, as it sets to offer an additional definition for a realm of science which is already existing, albeit at a smaller scale. Much like other advances in life sciences, this term too emanated from physics. As early as in 1959, Richard Feynman commented “there is plenty of room at the bottom,” years before the term

nanotechnology came in to existence (Feynman, 1960; Peterson, 2004). The molecular and intricate aspects of nanoscience were much later popularized by another physicist, Kim Eric Drexler, later in the 1980s (Drexler, 1981). Although much efforts have been made toward acceptance of scientific advances in the domain of nanotechnology, essentially these materials are not new but represent a divided state of matter in extremely small and alternate form (Fortina, Kricka, Surrey, & Grodzinski, 2005). This bears a two pronged advantage: first, the materials, particularly in the domain of life sciences, are already tried and tested and safety asserted; and second, the smaller size paradigm offers additional functionality (Cadden, 1987) and a new material of choice (Rao & Biswas, 2009). Essentially, nanotechnology would encompass anything that involves matter at atomic, molecular, and supramolecular scale both at two and three dimensions (Varnek, Fourches, Hoonakker, & Solov'ev, 2005). Nanotechnology includes both development of nanoscale devices as well as manipulation of nanoparticles (NPs). United States Environmental Protection Agency defines NPs as “an ingredient containing particles with at least one dimension that approximately measures 1–100 nm.” Similarly, European Union (EU) defines, “Nanoparticles is a natural, incidental, or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm” (EU, 2011). The NPs (<100 nm diameter at least in one dimension) have special physical, chemical, and electrical properties that are different from the bulk material (>500 nm), which provides wider scope of application of the same material. A special group of large NPs (100–500 nm) lies in between, showing distinct chemical or physical properties different from both the two extreme classes. The NPs can be naturally self-assembled, engineered under specific parameters, or simply naturally found without any specific reassembly (Monica & Cremonini, 2009). Based on structural properties, the nanomaterials may also be classified as nanocrystallines and nanostructures; the nanostructures may be polymeric (dendrimer, micelles, conjugates) or nonpolymeric [carbon nanotubes (CNTs), silica, or metallic NPs]. Based on the type and structure of nanomaterial assembly, the properties of NPs vary greatly. For example, based on the chirality of CNT, the final product may be a semiconductor or a metal (Saito & Zettl, 2008). The NPs can also be classified as 0D, 1D, 2D, and 3D particles on the basis of the dimension of the nanomaterial. The size of 0D NPs are <100 nm in all dimensions, whereas in 1D, one dimension must be <100 nm. Similarly in 3D, the nanomaterial has a length of >100 nm in all dimensions.

SUSTAINABLE AGRICULTURE AND NANOTECHNOLOGY

Agriculture is a fundamental operation for survival of mankind. Rapid expansion in population, boom in technological expansion for development

of essential as well as luxury products, and greedy demand of civilization have made agriculture gradually more exploitative, exhausting natural resources with a concomitant degradation of environment. Sustainable agriculture is a plausible solution to maintain productivity while protecting the environment. It is a system that maintains productivity and usefulness to society for indefinite period. Such system must be commercially competitive; otherwise, it will not be adopted. To achieve this, the system should be resource efficient, acceptable to society, and also should be environment friendly. Applications of nanotechnological advances are widespread in recent times in the realms of life sciences after innovations in material sciences have paved way for wider applications. Nanotechnology can be utilized intelligently to fit the basic structure of sustainable agriculture, particularly by contributing toward generation of resource-efficient technologies that are environmentally sound. The realm of application of nanotechnology in sustainable agricultural systems includes targeted delivery of chemicals, waste management, increasing hydrophilicity of soil, nanobiotechnological manipulation of genes and proteins, improvement of productivity by nutrient uptake at nanoscale, and many more exciting applications. With respect to agriculture and food safety, such novel applications are not only limited to crop productivity but also limited to holistic agriculture through livestock and fisheries management and waste management at farm scale and also by improving soil microbiome characteristics. Engineered NPs are mostly based on natural elements like carbon and silicon. Carbon being the most abundant of all elements both in all realms of the ecosystem, biotic and abiotic, nanostructured carbon and its derivative, namely, cylindrical CNTs and single-layered graphene, are suitable as biochemical sensors, carriers, and scaffolding structures that have huge applications in understanding plant's response, particularly under abiotic (temperature, pH, salinity, and nutrient limit) and biotic (insect–pest, pathogen, and weed) stresses. Similarly, other materials of choice, e.g., silica, clay particles, and various naturally derived materials ([Galbraith, 2007](#)), have been studied in length in potential agricultural sustenance mechanism. The nanolevel applications are not limited to carbon-based products only but extend also to other materials, including biocompatible, bioactive, and bioinert substances, some of which have antibacterial properties. Among the naturally occurring biological materials, amino acids chain with dendrimers and liposomes along with inorganic choices in the form of metals and metal oxides. Hybrid nanoentities with organic polymeric materials as well as peptide dendrimers can be the possible alternative. The choice of nanosubstances further reaches out to explore the applicability of biology-derived native-plant-derived products in their nanoform. There have been huge prospect in starch or cellulose-based NPs as possible nontoxic and sequentially sustained delivery. Such long-chain sugar NPs have been used in cell and pharmaceutical sciences for targeted drug delivery. Use of these biomolecular nanoentities holds great potential for agro-based industries.

APPLICATION OF NANOTECHNOLOGY IN SUSTAINABLE AGRICULTURE: CROP, LIVESTOCK, AND FISHERIES

Applications of Nanotechnology in Sustaining Crop Production

Nanotechnology has diverse potential applications in agriculture, from precision farming to nanobiotechnology. The usage of nanotechnology in agricultural practices is not new and can be traced back to use of clay and porous soil substitutes for water filtration and decontamination. Among all plausible applications, nanotechnology can find immediate application in crop protection followed by sustained nutrient release. As we are finding applications in nonrural agricultural applications, novel applications of NPs, such as use in soilless plant culture, would find increasing applications in near future. Nanocompounds, often the composite ones, offer significant advantage not only on plant growth mechanism and microbial population but also on soil microenvironment; thus, NPs can contribute to formulate a precise, efficient, and holistic agriculture in both rural and urban environment.

Nanotechnology to Boost Crop Productivity and Quality

A variety of NPs have been evaluated for boosting crop productivity, enhancing root and shoot formation, increasing root/shoot ratio, and enhancing fruit size and uptake of mineral nutrients (Nair et al., 2010). The NPs influence crop mineral nutrition by modifying enzymatic activity and modifying electron transport systems, exerting both positive and negative effects on crop growth and physiology. Plant uptake of NPs and tubes has primarily tested through seeds and seedlings (Lin & Xing, 2007). However, there have been efforts of improved uptake via roots (Karuppanapandian et al., 2011), pollen grains (Speranza, Leopold, Maier, Taddei, & Scoccianti, 2010), actual cellulose wall-protected plant cells (Liu et al., 2009), actual injected NPs (González-Melendi et al., 2008), or aerosol-mediated spraying onto seedlings and foliage (Anusuya & Sathiyabama, 2015).

Initial reports of NP uptake in plants described enhanced germination and seedling growth. Lentil (*Lens culinaris*) genotypes also exhibited improved germination and early seedling growth in response to SiO₂ NP application (Sabaghnia & Janmohammadi, 2014).

In soybean, Kole et al. (2013) reported up to 54% increase in biomass, 24% increase in water content, 20% increase in fruit length, 59% increase in fruit number, and 70% increase in fruit weight which ultimately resulted in 128% gain in fruit yield of bitter melon (*Momordica charantia*) on application of a carbon-based NP, fullerol [C₆₀(OH)₂₀]. The study also reported enhanced production of phytochemicals like cucurbitacin-B, charantin, insulin, and lycopene in response to fullerol application.

There have been concerted efforts in improving seed germination and plant growth by CNT uptake, particularly multiwalled CNT (MWCNT). Treatment of tomato seeds with 10–40 $\mu\text{g/mL}$ of MWCNT has shown dramatically increased rate of germination. The total weights of the seedlings as well as the root and stem lengths of the seedlings were also more in case of treated ones, compared to the untreated controls (Khodakovskaya et al., 2009). Root length was also found to be influenced by CNT treatment (Canas et al., 2008). Titanium oxide (TiO_2) NPs are another group of nanomaterial that has shown stimulatory effect on seed germination. TiO_2 -treated aging spinach seed showed 45% extra gain of chlorophyll-*a* which resulted in three times more photosynthesis, finally leading to 73% extra gain of dry weight compared to control over 30 days of growth period (Zheng, Hong, Lu, & Liu, 2005). Similar effects have been studied by using titanium nanoentities in wheat (Feizi, Rezvani Moghaddam, Shahtahmassebi, & Fotovat, 2012). Nano- TiO_2 increases uptake of water, oxygen, inorganic nutrients in the cell besides quenching reactive oxygen species (ROS), and breakdown of organic compounds. Many other metal NPs are also having similar properties. A study involving silicon (Si), copper (Cu), palladium (Pd), and gold (Au) NPs showed that Si and Cu NPs at high concentration, and Pd and Au NPs at low concentration possess germination enhancing ability of lettuce seeds (Shah & Belozeroval, 2009). Fluorescein isothiocyanate-labeled silica NPs also depicted induction of seed germination in case of rice (Nair et al., 2011). However, the response to germination appears to be variable in nature and requires more cellular level explanation based on the nanomaterial as well as the species of plant of choice (Yin, Colman, McGill, Wright, & Bernhardt, 2012).

Nanotechnology in Plant Disease Management

Nanotechnology also has several applications in plant disease management. The microbes are also a good source of biosynthesis of NPs, and a lot of research work has been carried out to find promising microbial sources of NPs. Of all NPs, silver NPs have been found to be most promising for disease control (Alghuthaymi, Almoammar, Rai, Said-Galiev, & Abd-Elsalam, 2015; Gong et al., 2007). The silver NPs are reported to interfere with oxygen metabolism process in bacteria and fungi, resulting in death of the microbial pathogen. In addition, NPs can destroy cellular DNA, increase production of ROS, interfere with electron transport system, and reduce nutrient uptake by microorganisms. Ag- SiO_2 NPs have been found very effective against various phytopathogenic fungi including *Alternaria solani*, *Botrytis cinerea*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Fusarium solani*, *Glomerella singulata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Phoma glomerata* (Jo, Kim, & Jung, 2009; Kim et al., 2012; Oh et al., 2006). Silver NPs

synthesized from *Serratia* sp. @10 µg/mL have been found to completely inhibit conidial germination of *Bipolaris sorokiniana* causing spot blotch disease in wheat (Mishra et al., 2014). Besides, ZnO and ZnTiO₃ nanopowders also exhibit high antimicrobial activity (Ruffolo et al., 2010). Application of nanosized silica-silver @100 ppm caused 100% growth inhibition of bacterial diseases like *Pseudomonas syringae* and *Xanthomonas campestris* pv. *vesicatoria* (Oh et al., 2006). To increase efficiency of silver NPs, graphene oxide has been used as support material to help accumulation of NPs on bacterial surface, which resulted in higher antibacterial activity against *Xanthomonas perforans*, the causal organism of bacterial spot of tomato (Ochoy et al., 2013). Among other NPs, nanoformulation of copper was highly effective against bacterial blight (*Xanthomonas oryzae* pv. *oryzae*), a serious bacterial disease of rice and mung bean leaf spot caused by *X. campestris* pv. *phaseoli* (Gogoi, Dureja, & Singh, 2009). Copper NPs were also found effective @200 g/L against various fungal pathogens (*A. niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *F. solani*, and *A. solani*) (Essa & Khallaf, 2016).

Nanopesticides

Application of nanomaterials in agriculture aims in efficient use of plant protection products and increases yields through optimized input management. The topic nanopesticide is gaining momentum in last two decades worldwide with exponential increase in the number of published papers and filed patent in this field. Compared to other fields, the definition of the term “nano” in pesticides is very loosely described. Nanopesticides include products that are designated with a “nano” prefix (e.g., nanohybrid, nanocomposite, nanoemulsions, nanodispersion) but do not belong to classical size range of nano, nevertheless, have properties associated with the small size.

Nanopesticides developed worldwide can be classified broadly into two groups. One group consists of nanomaterials having the potential to kill pests. These are mostly inorganic in nature and have pesticide effect. These groups consist of nanosilica, nanotitanium dioxide, nanozinc, nanosilver, nanocopper, nanoaluminum, etc. (Kah & Hofmann, 2014). Their efficacy has been tested and reported. For example, higher insect mortality with silica or aluminum NPs and antimicrobial properties of nanosilver or with nanocopper are well documented (Debnath et al. 2011; Kim et al. 2012; Mondal & Mani, 2012; Stadler, Buteler, & Weaver, 2010). These NPs may be popular in other sectors but do not have potential for large-scale agricultural applications. Toward a positive step, EPA granted a conditional registration for the first nanoproduct as “nanosilver pesticide” in 2011. The product was registered as an antimicrobial agent designed for use in textile industry but not in agriculture. But in the last decade or so, the second group, i.e., nanopesticidal formulation is gaining the thrust, and here,

organic-based nanomaterials are being used. Our group has done an extensive research on the use of nanoranged amphiphilic polymer on pesticide delivery through nanoencapsulation. Nanoformulations of imidacloprid, thiamethoxam, thiram, and carbofuran have greater residual life with sustained efficacy (Adak, Kumar, Shakil, & Walia, 2012; Shakil et al., 2010). Nanoemulsions of pesticides have got impetus due to their higher targeted activity. Nanoemulsions of neem oil, acephate, and glyphosate have better efficacy against their target pest compared to conventional formulations (Anjali, Sharma, Mukherjee, & Chandrasekaran, 2012; Jiang et al. 2012). Nanocomposites of pheromones, essential oils, have been developed (Abreu, Oliveira, Paula, & de Paula, 2012; Bhagat, Samanta, & Bhattacharya, 2013).

Proponents argue that pesticidal applications using nanotechnology promise efficient use of pesticide, due to their more precise and targeted nature and reduction in pesticide application rates and reduced losses. Smaller size of nanopesticides will have a higher total surface area, which offers overall greater contact with crop pests and higher absorption. Nanopesticide formulations increase the apparent solubility of poorly soluble pesticide molecule, release the active ingredient in a slow/targeted manner, protect from extraneous harsh environment, and prevent premature degradation losses. As such, nanotechnology is frequently portrayed as environmental benign (Kah, 2015; Liu & Lal, 2015).

Despite the potential of nanopesticides, it has not been popularized because of high cost of production. Till date, people may have accepted nanoproducts in cosmetics, food additives, or any other industrial application, but apprehension is very high for nanopesticides with respect to risk assessments. The properties of nanopesticides like enhanced transport, longer persistence, and higher toxicity may produce very different kinds of contamination in soil and other environmental matrices. Compared to inorganic nanopesticides, nanodelivery of pesticides with greener molecules should be considered. Biopolymer-based pesticides' delivery is being extensively investigated and has a bright future. But at present, the current regulations of different countries do not cover all the aspects of nanomaterials.

Abiotic Stress Tolerance

Under stress condition, NPs enhance germination and stimulate seedling growth possibly by increasing xylem humidity, water translocation, and/or better delivery of nutrients. Salt stressed seedlings of lentil showed significant improvement in germination percentage, shoot length, root length, seedling fresh weight, and seedling dry weight traits when treated with SiO₂ NPs (Sabaghnia & Janmohammadi, 2014). Silicon is known to enhance resistance to biotic stress, alleviate heavy metal toxicity, and provide mechanical strength to plant, though higher concentration of silicon may be toxic in acid

soils. Colloidal NPs are significantly less toxic than salt forms of different minerals. It has been observed that NPs of zinc, copper, and iron are up to 40 times less toxic than their salts (Syta, Novicka, Taran, Kalenska, & Ganchurin, 2010). Thus, application of NP-based formulations has better scope for productivity enhancement than salt-based applications. However, determination of appropriate concentration of NP solution is crucial. Taran, Batsmanova, Kovalenko, and Okanenko (2016) observed that presowing seed treatment @120 mg/L of nanoform biogenic metal (Ag, Cu, Fe, Zn, and Mn) colloidal solution enhances oxidative stress on soybean seedlings by 12%, whereas application @240 mg/L reduced oxidative stress by 19%. Such increase in oxidative stress at lower dose of NP colloidal formulation can be used to adapt the plant to be ready for abiotic stresses, as the level of expression of antioxidant enzymes also increases which are expected to contribute to higher resistance of the plant against stress. In addition to its effect on germination and seedling growth, the CNTs have also been found to induce tolerance to salt stress. MWCNTs showed positive effect on growth in NaCl-treated broccoli plants through increased water uptake and CO₂ assimilation (Martínez-Ballesta, Zapata, Chalbi, & Carvajal, 2016). In another study, Taran et al. (2017) observed that Cu and Zn NP colloidal solution can alleviate drought stress in winter wheat by inducing higher antioxidant enzyme activity. Increase in antioxidant enzyme activity resulted in decreased accumulation of thiobarbituric acid reactive substances, increased relative water content in leaves, and increased concentration of photosynthetic pigments.

Nanofertilizers

The NPs, owing to their high sorption capacity, surface area, and capacity of controlled release of mineral nutrients, are considered as “smart” carrier for efficient delivery and release of fertilizers. The encased chemicals are better protected against natural extremities. It has been perceived that such modulated delivery would not only provide nutrients at targeted locations but also improve plant metabolism in a sustainable manner (Nair et al., 2011). Mesoporous aluminosilicate-based NPs have high potential for controlled delivery of macro- and micronutrients in soil. A nanosized Mn-carbonate hollow core–shell system was found to favor regulated release of Zn more slowly than the ZnSO₄ salt, which resulted in better growth of rice under both submerged and aerobic conditions. Use of SiO₂–TiO₂ NP combinations as fertilizer has also improved nitrate reductase activity in soybean, resulting in better nutrient uptake. Iron oxide NPs (Fe₂O₃ NP) are another potential candidate as nanofertilizer. Maghemite (γ-Fe₂O₃), an iron oxide NP with 20 nm average particle size, was used as fertilizer on peanut (*Arachis hypogaea*), and plant height in all the nanofertilized treatments

was found to be higher than chelated iron (Rui et al., 2016). Both the treatments increased chlorophyll content, increasing photosynthetic activity. In addition, concentration of growth promoting hormones like gibberellins and zeatin-riboside increased considerably and concentration of abscisic acid decreased considerably in nanofertilized plants. It was also observed that when applied as nanofertilized, iron adheres better to the soil particles, which increases the chance of availability of iron in plants. However, nanoencapsulation is still less efficient than microencapsulation of fertilizers; thus, more researches are needed to make nanoencapsulated fertilizer delivery cost-effective.

Coating of fertilizers with NPs for developing slow release fertilizers is another potential avenue to enhance fertilizer use efficiency in plants. A thin protective layer around the fertilizer capsule helps to protect the capsule, allowing more control over nutrient availability to the root rhizosphere. Besides, fertilizers can also be trapped into a nanoporous material and applied to the plant for controlled release. Coating of urea with NPs has shown to help in slow release of urea.

Improvement of Photosynthetic Efficiency

An associated mechanism of seed incorporation of nanoentities is the ability in the seedling or plant to modulate photosynthetic ability. As an inherent ability of NPs in increasing surface-area-to-volume ratio, the improved ability of small rods or particles in improving the light reaction phase of photosynthesis. There have been studies elucidating the enhanced ability of NPs in absorbing or responding to particular wavelengths of light, thereby highlighting their optical potential, a factor very crucial in light-dependent stage of photosynthesis (Hills reaction) often responding to either the photosystem II or the photosystem I (Noji et al., 2011). This ability to respond to particular wavelength could effectively mimic solar photovoltaic like ability in green leaves, otherwise nature's most efficient solar energy convertors. NP incorporation into plant models can improve photosynthetic efficiency both at light-dependent as well as light-independent reaction. For example, nano-TiO₂-treated aging spinach seed showed 45% extra gain of chlorophyll-*a* which resulted in three times more photosynthesis, finally leading to 73% extra gain of dry weight compared to control over 30 days of growth period (Zheng et al., 2005). Nano-TiO₂ increases uptake of water, oxygen, inorganic nutrients in the cell besides quenching ROS and breakdown of organic compounds. But the most significant effect of TiO₂ NPs is that it increases light absorbance and activate Rubisco activase which in turn maximizes the total photosynthetic yield. This innovation can help improve agricultural efficiency in the long run. This ability can translate to higher production of proton carriers which can effectively carry out improved sugar production phase

in the Calvin cycle or dark phase (Sharma et al., 2012). This intervention can effectively not only make the green leaves photosynthesize better or faster but also produce faster or improved food for storage, thereby improving the sink mechanism of prepared sugar.

Water Retention and Management

A ubiquitous concern among all sustainable agricultural practices is the efficient utilization of water and prevention of irrigation spillage. Nanomaterials, primarily in the likes of carbon nanotubes, have exhibited increased retention of water (Khodakovskaya, De Silva, Biris, Dervishi, & Villagarcia, 2012). This phenomenon can be attributed to improved xylem vessel mechanism, plant metabolism and physiological conditions, and proper utilization and reinforcement of sieve tubes concerted with increased sink conditions (biomass, fruits, and other storage spaces). As most nanoentities like nanotubes are dispersible in water and not completely soluble, the retention of water is hence remarkably improved in these plant systems. Increased water uptake and concomitant growth enhancement due to MWCNT treatment were also demonstrated in pumpkin (Corredor et al., 2009) and broccoli (Martínez-Ballesta et al., 2016). In both the cases, accumulation of CNTs was clearly visible inside the plant cell through transmission electron microscopy which shows that CNT penetrates the plant cell and works as water transporter. MWCNTs serve as nanosized water channel where water moves through the hollow core of the MWCNTs as a continuous column, due to cohesive pull imposed of one water molecule upon others and no adhesion between the water molecule and carbon atoms (Khodakovskaya et al., 2012). With respect to groundwater and stabilization of the soil horizons, applications of nanotechnology can reap great benefits, leading to reduction in ecotoxicity. The work on decontamination by NPs started in the early 1990s, by conceptualizing use of zero valence iron in permeable reactive barriers. Studies showed that reactivity of zero-valent iron NPs was 100 times more than its macroscale form. Many NPs including nanotubes and carbon fiber, nanometal oxides, nanoenzymes, and bimetallic NPs have been found to be effective for decontamination of groundwater.

Nanobiotechnology: Application in Agriculture and Plant Science

Nanobiotechnology, in effect, deals with manipulating biological systems using nanodevices in such a way that has never been achieved earlier. In simple terms, manipulating cellular architecture, metabolism or genetic makeup of an organism by applying nanosized particles is considered as nanobiotechnology.

Use of NPs to Deliver Molecular Cargos Inside Plant Cell

Compared to animal cells, transport of molecules into plant cells is more complicated due to the presence of cell wall. It acts as an extra barrier to the process of transport in addition to cell membrane. Owing to the unique ability of NPs to easily penetrate plant cells, these can serve as ideal vectors to deliver several molecular cargos including DNA. Due to very high aspect ratio, CNTs, in effect, function as nanosize niddles and hence serve as an ideal material for this purpose. Method of CNT uptake by the plant cell is not very well elucidated, but experiments suggest that endocytosis is the most probable mechanism for it (Liu et al., 2009). Once taken up by the cell as bundles, discrete CNTs escape out the endosomes into the cytoplasm by endosomal leakage (Yaron et al., 2011). Apart from this, few discrete CNTs can even penetrate the cell membrane directly and enter the cytoplasm of the cell (Yaron et al., 2011). Once inside the cytoplasm, it can penetrate and enter any cell organelle including nucleus (Mu, Broughton, & Yan, 2009). But, unlike water molecules which move through the inside hollow core of the CNTs, other molecular cargoes like dyes and nucleic acids are carried as surface adsorbed material at the outer surface of CNTs. Single-walled CNTs (SWCNTs) are preferred over MWCNTs for this purpose as it has broad outer surface area. CNTs don't have good solubility in water and also being hydrophobic in nature attaching molecules by electrostatic force is difficult. So, it need surface fictionalization treatments which increase the acceptance of molecular cargos by the CNTs and also enhance its water solubility. Once the cargoes are attached to the CNTs, the CNTs can penetrate the wall and membrane of the cell to deliver those cargoes inside (Liu et al., 2009; Mu et al., 2009). Apart from this, an interesting observation was made also by Liu et al. (2009) while working with *Nicotiana tabacum* L. cv. Bright Yellow (BY-2) cells. As they delivered SWCNTs coated with fluorescent dye Fluorescein IsoThioCyanate (FITC) (FITC/SWCNT) and with FITC-attached single stranded DNA (ssDNA) (FITC-ssDNA/SWCNT) separately, confocal microscopy revealed preferential accumulation of FITC/SWCNT-conjugate at vacuoles, whereas FITC-ssDNA/SWCNT conjugates at cytoplasmic strands. The reason behind this site specific accumulation was not understood. But one thing came out of this study is that once this mystery is unfolded, CNTs can be used to deliver molecular cargos at specifically desired sites. Apart from this, spraying and/or injection of CNT-coated iron nanoparticles also demonstrated long distance transport and magnetic field induced accumulation of NPs away from the site of application in standing pumpkin plants (Corredor et al., 2009). This finding opens up the unique possibility of developing generalized *in planta* transformation procedure which, if developed, will save the painstaking tusk of tissue-culture-mediated regeneration of transformed plant materials. For present, compared to the existing physical delivery methods available in plant cells, such as the

particle bombardment, electroporation, and microinjection, the NP-based delivery strategy seems to be advantageous because of its operational ease and higher efficiency. Apart from CNTs, mesoporous silica NPs can also be utilized to deliver biomolecules like DNA and protein inside plant cell (Torney, Trewyn, Lin, & Wang, 2007).

Use of NPs to Deliver Genetic Material and to Modulate Gene Expression

The ability of NPs to deliver molecular cargo including nucleic acids can be effectively utilized to deliver genetic material to the target cells and thus induce expression of specific genes at desired sites. Surface-functionalized vertically aligned carbon nanofibers are able to attach plasmid DNA as molecular cargo and deliver it into the target cell where the exogenous gene carried by the plasmid was found to be expressed normally like other conventional transient expression systems (McKnight et al., 2003; McKnight et al., 2004). Liu et al. (2008) and Liu, Yuan, Yue, Zheng, and Tang (2008) developed a unique fluorescent-labeled starch-NP-based transgene vector which is able to penetrate plant cell wall, cell membrane as well as nuclear membrane to deliver its molecular cargo. Ultrasound was used to generate transient pores to deliver the loaded NPs. Fluorescent label attached with the NPs allowed visual tracking of the transgene inside the cell. MSN NPs surface functionalized with triethylene glycol were used to deliver plasmid containing green fluorescent protein inside the plant cell which showed normal expression (Torney et al., 2007). Recently, fluorescent conjugated polymer NPs were used to deliver siRNAs in *N. tabacum* L. cv. BY-2 protoplast and posttranscriptional gene silencing was achieved for two of the cellulose synthesis pathway genes, viz. *NtCesA-1a* and *NtCesA-1b*. Another unique advantage of NP-based gene delivery is that, if needed, the delivered DNA can be tightly attached to the NP so that it does not get detached from the NP (Nair et al., 2011). In that case, the delivered gene will be only expressed transiently at the delivery sites but will not integrate to the genetic material of the recipient. By this way, site specific expression of desired gene can be achieved like in case of gene therapy by avoiding the step of stable transgenic development. Apart from these, simply treating plant cells with specific NPs can also upregulate or downregulate specific group of genes. For example, treatment of spinach with nano-TiO₂ can upregulate superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase in chloroplast (Liu et al., 2008; Liu, Yuan, et al., 2008) besides inducing Rubisco activity. Similarly, nano-Al₂O₃ treatment of tobacco has shown induction of miR395, miR397, miR398, and miR399 microRNAs (Burklew, Ashlock, Winfrey, & Zhang, 2012).

Nanomaterial Synthesis From Plant Sources

Biological synthesis of NP is gaining importance in recent times as the physical and chemical methods of NP synthesis are considered to be environmentally hazardous, apart from being a costly affair too. Under this scenario, use

of plant as a factory to synthesize NPs provides an easier, cheaper, and eco-friendly alternative to synthesize nanomaterials. The ability of production of metal NPs by plants depend on the reduction potential of the metal cation and the reducing power of the plant cell sap or extract which in turn depend on the amount and combination of different polyphenols in it. The site of accumulation of nanoparticles also varies plant to plant. Gold NPs were found to be synthesized efficiently in alfalfa plants and *Sesbania* seedlings when the plant were grown in media simply containing Au-salts (Gardea-Torresdey et al., 2002; Sharma et al., 2007). Similarly, silver NPs were obtained using some metallophytes like *Brassica juncea*, *Medicago sativa*, *Alfalfa sprouts*, etc. (Gardea-Torresdey et al., 2002; Harris & Bali, 2008). Rapid synthesis of silver NPs were also achieved simply by adding the leaf extract of plants like pine, persimmon, ginkgo, magnolia, and platanus. Among these, more than 90% conversion from AgNO_3 to silver NPs were obtained just by adding leaf extract to the AgNO_3 solution and heating the mix at 95°C for 11 minutes (Song & Kim, 2009). Biomineralization of copper by semiaquatic plants at the interface of soil–root interface, aided by endomycorrhizal fungi is also reported to produce Cu NPs.

Nanotechnology in Reducing Postharvest Loss

Nanotechnology finds considerable applications in packaging and preservation technologies for reducing postharvest loss of food products. The procedure involves sensory coating which might change in color or similar visible parameters based on the quality of the content. Separation techniques, filtration or removal of effective parts of agricultural or livestock product, and most effectively encapsulation or wrapping procedure can provide effective alternative not only to increase shelf life but also to value addition procedure to the food item procured (Rajkumar, 2006). The product packaging is not limited to solid or processed foods itself but also to liquid beverages as well as nanocomposites in the likes of nanoceramics offer a better shelf life and what is believed to be an improved after taste. Thin film of nanoproducts can offer a protective layer retaining moisture and preventing biofouling or spoilage. Incorporation of relatively cheaper thin carbon film or proven antibacterial materials like zinc oxide, etc. would enable a more effective packaging procedure which not only increases the shelf life of fresh produce but provides environmentally compatible nontoxic substitutes.

Livestock and Agricultural Animal Husbandry

Nanochips have been employed for sensing and monitoring livestock and poultry health, physiology and behavior (Kannaki, 2006b). This also enables estimating the quality of produce, both meat as well as animal products by close monitoring. For whole animal or animal product export-oriented economy, these smaller details in animal, bird, fish tracking system improve the

quality of produce both qualitatively and commercially at an international market (Singh, 2012). The quality of meat produce and possible contaminants (inclusive chemical fertilizers and pesticides) which can lead to allergic response could be tracked thereby also preventing spoilage of the protein produce in a relatively cheaper and cost-effective manner (Rajkumar et al., 2006). There has also been significant ideation in artificial meat production using nanotechnological methods (Moraru et al., 2003). Among other animal produce, milk production accounts for a large share in both economy and volume. Improved methods to prevent microbial spoilage, improved pasteurization and better preservation of nutrients and ascertain quality of milk solids are possible avenues of nanotechnological advances (Kuzma, 2006). Another widespread animal produce avenue is in egg production where nanocarrier mediated nutrient fortification and prevention of pathogen infection thus determining their quality using nanooptics (Kannaki, 2006a).

Fishery and Aquaculture

The aquaculture domain is a major area where nanotechnological advances can turn out to be effective. The benefits and possible advantages are numerous; the process would help procure high quality fish and sea food for consumption purpose offering targeted dosage of essential proteins and oils. In addition, sustainable practices would help in coordinating crop farming practices to fish farming as well thereby conserving water and maximizing resources. Smart sensor and drug delivery devices can greatly promote nutrition and health along with prevention and detection of pathological situations in the fish populace. Along with microbes like bacteria, water culture also involves effective sensing of algae and other biological entities which can improve fish quality. Lanthanum-based NPs can prevent alga population in water by removal the phosphate compounds (Zhang, Shen, Shan, Mei, & Wang, 2011) often even leading to eutrophication remedies (Copetti et al., 2016). NP-encapsulated fish feed has also been tried effectively without any visible side effect. In addition, the cyanobacterial population have also been mitigated by phosphorous binding clay which promotes flora and fauna of the region improving water quality, plankton growth, and fish growth (van Oosterhout & Lüring, 2013). For effective controlling of algae, individual cations such as nanohybrid mixes have been used as a matrix support material (Ivanova, Toncheva-Panova, Chernev, & Samuneva, 2008).

Biological Waste Management Through Nanotechnology for Sustainable Agriculture

Multiple solid-waste management has been a major hazard in agriculture remains. These in turn affect water management and soil contamination procedures. Nanotechnology can offer sustainable solutions in waste

management of agricultural process and produce. The substitution of existing material choices to bionanomaterials and nanobiomimics can effectively make the process of waste management more sustainable. For example, natural fiber material (spider web, silk, and hemp) inspired nanofibers are being employed to make absorbable materials which can degrade effectively without leaving naturally toxic remnants or residues.

In a method similar to biosimilars and biomimics, nanoproducts can be developed in similar dimensions of natural degradable fibers in the likes of cellulose. Some of these biorenewables are naturally degradable like green tea which also offer quenching of ROS like theranostic properties (Kim, Lee, Song, & Kang, 2015). These naturally inspired nanofibers can be potentially used as delivery and scaffold structures for sustainable farming practices and they degrade effectively with time. In addition, with advances in biomass procurement and trade of biomass across geographies, these nanocomposites can enrich the biomass production and quality as well.

CONCERNS ABOUT NANOTECHNOLOGY

Legal and Regulatory Concerns

Nanoentities are defined by nonhomogeneous legal regulations. While in countries like India, Thailand and other developing nations, nanoentities or new forms in nanoscale of a known (or protected) compound providing additional efficacy or applicative role cannot be patent protected and are labeled evergreening practices. Clauses akin to the Section 3d in the Indian Patent Act have redefined the scientific merit of a proven size or shape or physical parameter exclusivity of materials of choice (Reddy, 2008). This clause limits the applicability of otherwise common or already discovered materials even though supported with sound scientific evidence thereby compounding such enhanced role as frivolous. So, if usage of clay or similar forms of silicate as plant moisture retainer is common, a NP form of clay which prevents a higher amount of moisture loss would come under the purview and could not be patent protected, hence ruling out any potential research and development around that domain. These laws are a major deterrent to practices of sustainable agriculture which could possibly have benefitted greatly through nanotechnological approaches.

The international legal regulatory authorities often do not recognize naturally occurring nanoentities and synthesized or created nanoentities as different forms. Taking the example of the EU (Restuccia et al., 2010), the motive of inception of a nonentity composite does not discriminate between thought after scientifically rationalized idea into an actual working product from something which is rather naturally found or accidentally created (Bowman & Hodge, 2006). As efforts in research and development improve in the realm of nanotechnological approaches to solve agricultural issues, the

regulatory and environmental framework also would improve and strengthen with time (Reynolds, 2003). Unlike FDA approval protocol for drugs and clinical devices where nanoentities are used, such regulations are highly missing in plant applications.

Socioeconomic Concerns

The socioeconomic concerns for nanotechnological interventions to traditional agricultural practices with a sustainable paradigm do not differ much from the usage of the same chemical, biologics or practice that is already in operation. The nanotechnological advances are not solely limited to commercial benefits or higher return for both the farming and industrial communities, but a sustained nature friendly manner in a proposed plan akin to the concerted efforts in the pharmaceuticals domain. The legalization of nanotechnological products would allow effective labeling and market detailing procedures so individuals can make an informed choice. Retaining of half time and efficacy enhancement of agrochemicals and biologics for plant produce can be significantly improved using NPs and fibers. This can help alleviate longer soil and water uniformity in distribution and prevention of loss. However, the potency and time resolve efficacy still needs to be determined. Natural products and formulations in the nanoscale have been in usage since long. Products like nanoscale clay, silica, polymeric substances, carbon, and its substitutes—all have been in application since decades. The potential is huge, hence ascertained the enormous leap and bounds of the increasing number of patent applications in the last few decades. This is important as patent procedures for generic agricultural applications pertaining to sustainability are not as widespread as other sectors. There is a strong possibility that there might be an infringement clause or overlap of invention disclosure for existing patents and the newly founded applications of the same materials in nanoscale, thus enabling a host of infringement legalities.

Toxicological and Environment Safety Concerns

Rapid expansion in nanotechnology application also brings out the safety concerns of the NPs in biological system. Many NPs are toxic to microbes; thus, uncontrolled applications of NPs as nanopesticide and nanofungicide formulations may result in the environmental pollution and degradation caused by the chemical pesticides. The buildup of these NPs in soil and entry into food chain thus should be well investigated to design safety protocols for mass release of nanomaterial-based technologies. The uptake and translocation of NPs in crop plants also need to be investigated critically. A number of studies have reported dose-dependent toxicity of NPs in crop plants (Li et al., 2015). For example, pure aluminum NPs inhibited root growth in several crop plants like corn, carrot, cucumber, carrot cabbage, and soybean.

Alumina (Al_2O_3) NPs work as an environmental pollutant and it hampers plant growth. Tobacco seedlings showed gradual and significant decrease in average root length, biomass, and leaf count with increased concentration of Al_2O_3 NP exposure (Burklew et al., 2012). Zinc- (Zn) and zinc-oxide (ZnO)-based NPs also depicted inhibitory effect on physiological phenomenon like seed germination and root elongation in several plants like rye grass, radish and rapeseed (Lin & Xing, 2007). Copper NPs are reported to have cytotoxic effect on mung bean whereas silver NPs on zucchini and onion. Many studies also reported cytotoxic effect of MWCNT at higher concentration on several plants including rice and *Arabidopsis* (Nair et al., 2010). These suggest the need of carrying out experiments in order to determine the environmentally safe dose of NPs. For example, nano-ZnO, assimilated through root from soil, accumulates in edible parts of soybean plants, affecting food quality, whereas another NP, nano-CeO₂ considerably reduced nitrogen fixing potential and yield of soybean plants (Priester, Ge, & Mielke, 2012). The size exclusivity of nanoentities can also potentially offer hazard condition, for both plant and animals. Being extremely tiny in nature these particles can block plant openings like stomata which can radically affect plant physiology and morphology. As nanomaterials can easily overcome barriers, their efficacy, retention, and degradation are studied closely (Vishwakarma, 2010). Reproductive parts in the likes of pollen can be severely affected by such NPs interface thus affecting movement and uptake of water, minerals, sugar, and its intermediates produced. Very similar effect would happen in animals as well, and the hazard role would be more enhanced in vasculature and blood vessels. To make matter worse, the basic nature of these nonentities can lead to incorporation of these effects to the genomic content and lead to genetic toxicity, along with inflammatory, respiratory, and hematological conditions. Environmental effects in soil and water retention also exists largely and these are more pronounced over time. According to FAO/WHO, even though many progresses have been made in NPs risk assessment, but data gaps exist worldwide (FAO/WHO, 2013). Hence, usage of nanoentities in agricultural chemicals and biomolecules should be approached with precaution, suggestively based on different existing regulatory frameworks enabling this rapidly emerging technology, significantly improving sustainable agricultural (Watson & Janus, 2011) with adequate time resolved trial and proper validation.

FUTURE OUTLOOK

NPs have high application potential in sustainable agriculture using suitable regulatory framework as long as it is environmentally safe and have economic viability. A concerted effort to this regard can prove to be effective in redefining affordable agricultural productivity. The increasing amount of attention and interest in nanotechnology indicate a significant paradigm

shift toward sustainable precision agriculture. Concerted approach and targeted output, with policy support, is however, extremely limited. Because the science is size exclusive, this very size reliability can make an otherwise improved efficacy entity into a toxic substance. Although ethical, toxic, regulatory, and policy concerns around nanotechnological approaches have to be carefully considered, this also enables the discipline to evolve much more comprehensively. Hence, improved research endeavor, ably supported by funding agencies, should work to remove the potential bottlenecks of nanotechnology applications by developing environmentally safe nanoagricultural systems.

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Chapter 11

Biosafety for Sustainable Agriculture

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INTRODUCTION

Every plant has a specific genetic constituent and morphology in the nature. However, the genetic makeup has been manipulated by plant breeder and farmers for more than 10,000 years. The change in the genetic consistency of most crops without any knowing what is heredity has been applied earlier was not known. After rediscovery work of Mendel genetics as biological sciences, plant breeders and as well as genetic engineers now know how

plant exist their genetic behavior in the field. Unlike conventional plant breeding, modern plant genetic engineering does not rely on making crosses in between two parents. Modern molecular biology tools have been coupled with GE or genetic modification of the plants for their specific traits. Recombinant DNA technology, or GE, is an additional and specific form of biotechnology which allowed breeder to transfer known, desirable genes into crops for specific traits, instead of moving large groups of mostly unknown genes into crops, as in common traditional breeding.

The genetically modified (GM) crops have reflected the possibility of covering large proportion of the food crops cultivated by growers in the coming future due to limitations of conventional breeding for attaining the desirable traits. Controversies around transgenic crops keep revolving including their proponents and opponents both in the society and scientists being viewed with suspicion by many, whereas others see it ethically sound (Robinson, 1999). In current scenario, people today want to get maximum benefits without changing aspects of technologies with completely risk free. Thus, the degree of risk involved plays a major role in social acceptance of GE and genetically transformed crops (Mannion & Morse, 2013). Therefore, transgenic technology, so-called synthetic biology, continuously keeps focus on the advantages and disadvantages of GM crops and has become a highly debatable and burning issue (Benner & Sismour, 2005). The GM technology in each aspect affects safety, trade-related aspects, and ethical aspects of consumers, researchers, and environmentalists which automatically explain to necessitate the need for implying regulation of GM products (Francis, 2006). Most of the countries have participated and ratified after signing the Convention on Biodiversity (CBD), Cartagena Protocol. There is other regulatory legislation to exploit huge theoretical as well as probable potential from modern biotechnology applications, whereas simultaneously safeguarding against potential risks has been signed by the different countries.

TRADITIONAL PLANT-BREEDING APPROACHES VS NEWLY ESTABLISHED TRANSGENIC TECHNOLOGY

Traditional plant-breeding program has been an incorporated part of varietal development and improvement. Plant breeders have contributed enormously in the improvement of crop grain production as well as quality. In addition, it affords enclosure of all the recently arriving plant-improvement techniques and supports novel approaches such as transgenic varieties that have the potential pioneer traits of attention from different crop variety or different plant species (Hull, 1945; Dudley & Lambert, 2004). Though conventional plant-breeding techniques are time and labor consuming to breed better varieties, traditional breeding approach has crossing, backcrossing, and re-crossing procedure in the selected plants over numerous generations and observance eye for desirable traits. Once a prospective variety is developed, the innovative variety

must be assessed under different environmental circumstances to monitor whether the cross introduced any disadvantageous traits, if any. However, the benefit of transgenic technology is that it permits introducing a concern gene or cluster of genes for necessary traits and estimating its outcome in early generations. Concurrently, it has also a potential to determine the presence of undesirable gene introduced at the same occasion. Therefore, transgenic technology can be used to harmonize the potential of traditional plant breeding to create superiority crops with superior exactitude. In totaling, transgenic technology not only is bounded to gene transfer from same species but can introduce characters from other species. At present, several transgenic crops expressing herbicide or pesticide-resistance genes make the crop tolerant against the target stress (Key, Ma, & Darke, 2008).

Genetically Modified Crops

Genetically modified crops, often known by the acronym “GM crops,” are typically established with changeable emotions worldwide. GM crops are crops of which, the DNA has been modified using various GE techniques. The GM crops are a target to set up a new characteristic to the plant which does not occur in nature in particular plant species e.g., in food crops, various pest-resistant, abiotic stress-resistant, spoilage-resistant, chemical-resistant genes have been incorporated along with those genes which have improved the nutrient profile of the crop, respectively (Boyle, 2011). The modification for gene transfer most commonly includes the transfer of gene or region of genome of interest from a different species (bacteria, animal, or plant). Moreover, after discovery of Bt toxin from *Bacillus thuringiensis* bacterium in 1901, it is frequently used as a donor insecticidal protein. Furthermore, the discovery of *Agrobacterium tumefaciens* in 1907 offered a unique tool for transfer of the *Bt* genes to crops, ushering in a new era of gene transfer across plant species, a process that became clearer following Watson and Crick’s unraveling of the DNA structure in the 1950s. Biotechnology thus provides a complementary approach to conventional breeding methods.

According to USDA (2007a, 2007b), agricultural biotechnology offers an additional tool for increasing crop productivity, especially when conventional methods cannot deliver on breeding targets. This offers a great breakthrough in Africa toward advancing even faster food security and poverty eradication. A number of studies have been done to assess the impact of GM crops on farm productivity in the developing countries (Huang, Hu, Rozelle, & Pray, 2005; Zilberman, Ameden, & Qaim, 2007). There is unanimous agreement that biotechnology is indeed an important tool for increasing farm productivity for the smallholder farm sector. A study by Subramanian and Qaim (2009) provides empirical evidence that production of *Bt* cotton has direct and spillover positive socioeconomic effects on all types of rural households

through improved yields and increased employment. These important findings point to the role that GM crops can play in solving poverty and development issues (Toenniessen, O'Toole, & De Vries, 2003).

Genetically Modified Crops/Transgenic Crops Status

In 1983, the first successful GE of plant was reported when tobacco and tomato were transformed. The following section briefly discusses with the different transgenic in the world as well as in India.

Global Status of Transgenic Crops

In 2003, 7 million farmers of 18 countries grown transgenic crops and more than 85% of them were resource poor farmers and out of these 85% farmers were from the developing world. Almost one-third of the global biotech crop area was grown in the developing countries (James, 2015). A decade after GM crops were introduced into the world, their production has grown to about 125 mha globally (Table 11.1). The GM crops that are produced on a commercial basis have been limited to soybean (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), maize (*Zea mays* L.), and oilseed rape (*Brassica napus* L.). These four crops have been transformed for the two traits of herbicide tolerance and insect resistance (James, 2001, 2002, 2014, 2015). However, papaya (*Carica papaya* L.) is one of the transgenic crops with virus resistance that has been commercialized. Papaya with resistance to papaya ring spot virus (PRSV) is now grown on a commercial basis by farmers on the Hawaiian islands, where GM technology was used to save the local papaya industry from total collapse due to infection by PRSV (Ferreira et al., 2002).

In 2008, China, Paraguay, and South Africa cultivated GM crops on an area of over 1 mha, and in the same year, Bolivia, Egypt, and Burkina Faso cultivated GM crops for the first time. So far, the Philippines approved 21 transgenic varieties for food, feeds, and processing. These include *Bt* maize, herbicide-tolerant maize, rice, soybean, canola, potato, cotton, sugar beet, and alfalfa. India has also approved GM cotton which is at present being cultivated on about 1.5 mha. The other crops (eggplant, rice, cauliflower, tomato, okra, potato, and mustard) are under trial for potential release (ASSAF, 2010). China has about 3.3 mha under GM-crop production—the Chinese government has committed vast resources (US\$1.4 billion) for the development of agricultural biotechnology and has established more than 100 biotechnology laboratories, signifying intent and commitment by the country to use biotechnology to address its food security challenge (James, 2009). Two Latin American countries, Argentina and Brazil, are following the global giant in GM-crop production with 21.0 and 18.5 mha, respectively, mainly *Bt* maize and Roundup Ready soybean. Other countries

TABLE 11.1 Global Status of Transgenic Crop and Their Production.

Sr. No.	Country	Area (million ha)	Rank	2014	2015	
1	USA*	73.1	1	73.1	70.9	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash
2	Brazil*	42.2	2	42.2	44.2	Soybean, maize, cotton
3	Argentina*	24.3	3	24.3	24.5	Soybean, maize, cotton
4	India*	11.6	4	11.6	11.6	Cotton
5	Canada*	11.6	5	11.6	11	Canola, maize, soybean, sugar beet
6	China*	3.9	6	3.9	3.7	Cotton, papaya, poplar, tomato, sweet pepper
7	Paraguay*	3.9	7	3.9	3.6	Soybean, maize, cotton
8	Pakistan*	2.9	8	2.9	2.9	Cotton
9	South Africa*	2.7	9	2.7	2.3	Maize, soybean, cotton
10	Uruguay*	1.6	10	1.6	1.4	Soybean, maize
11	Bolivia*	1	11	1	1.1	Soybean
12	Philippines*	0.8	12	0.8	0.7	Maize
13	Australia*	0.5	13	0.5	0.7	Cotton, canola
14	Burkina Faso*	0.5	14	0.5	0.4	Cotton
15	Myanmar*	0.3	15	0.3	0.3	Cotton
16	Mexico*	0.2	16	0.2	0.1	Cotton, soybean
17	Spain*	0.1	17	0.1	0.1	Maize
18	Colombia*	0.1	18	0.1	0.1	Cotton, maize
19	Sudan*	0.1	19	0.1	0.1	Cotton
20	Honduras	<0.05	20	<0.1	<0.1	Maize

(Continued)

TABLE 11.1 (Continued)

Sr. No.	Country	Area (million ha)	Rank	2014	2015	
21	Chile	<0.05	21	<0.1	<0.1	Maize, soybean, canola
22	Portugal	<0.05	22	<0.1	<0.1	Maize
23	Vietnam	<0.05	23	<0.1	<0.1	Maize
24	Czech Republic	<0.05	24	<0.1	<0.1	Maize
25	Slovakia	<0.05	25	<0.1	<0.1	Maize
26	Costa Rica	<0.05	26	<0.1	<0.1	Maize
27	Bangladesh	<0.05	27	<0.1	<0.1	Cotton, soybean
28	Romania	<0.05	28	<0.1	<0.1	Brinjal/Eggplant
	Total			181.5	179.7	

*Represent the 19 different countries all over the world growing 50,000 hectares or more GM crops
Source: Adopted and modified from James, C. (2015). 20th anniversary (1996 to 2015) of the global commercialization of biotech crops and biotech crop highlights in 2015. ISAAA Brief No. 51. Ithaca, NY: ISAAA.

producing GM crops (*Bt* cotton, *Bt* maize, and Roundup Ready soybean) in the region are Paraguay, Bolivia, and Uruguay (Otiman, Badea, & Buzdugan, 2008; Rodriguez-Cerezo, 2009).

Total land under GM crops cultivation in 2011 was 159 mha which is only 3% of the world's agricultural land (Adenle, 2011). Of this, 23% was maize that had been GE to possessing insecticidal δ -endotoxins from the soil bacterium *Bt* conferring resistance against European corn borer (*Ostrinia nubilalis*) (James, 2002; Singh et al., 2014). In India, genetically engineered *Bt* cotton was given conditional clearance to Monsanto and Mahyco for commercial planting of the cotton in four states of southern and central India (Chopra & Kamma, 2012). In 2013, 91% of global GM production is in the United States, Brazil, Argentina, India, and Canada. Despite the hype that GM is the fastest adopted technology, even these five countries use conventional farming in majority of agricultural land. The global share of transgenic crops is already considerable; 36% of all soybean, 16% of cotton, 11% of oilseed rape, and 7% of maize were transgenic in 2000 (James, 2001). Though the industries promoting transgenic food crops and products are required to assess

the potential environmental hazards and health consequences of their products, the results seem to be not much effective (Purrinton & Bergelson, 1995).

The year 2015 is considered as the 20th year for commercialization of biotech crops. From the initial planting of 1.7 mha in 1996 to 179.7 mha in 2015, it was a remarkable 100-fold increase since the start of commercialization. Thus, biotech crops are considered as the fastest adopted crop technology in the history of modern agriculture. In 2015, ~18 million farmers planted biotech crops in 28 countries, wherein over 54% or about 97.1 mha were planted by small and resource-poor farmers from the developing countries. The highest increase in any country, in absolute hectareage growth was Brazil with 2 mha (James, 2015).

The relative area of biotech crops in the industrial and developing countries from 1996 to 2015 is continuously increased. In 2015, for the fourth year, more than half (54%) of the global biotech crop area of 179.7 mha, equivalent to 97.1 mha, was grown in 20 developing countries. In 2015, year-to-year growth was higher in the developing countries at 0.9 mha (1%) than in industrial countries which were reduced by 3% (2.7 mha). This is attributed to the increase in soybean plantings in Brazil and Argentina, as well as cotton plantings in Pakistan, Myanmar, and Sudan. Thus, year-to-year growth was significantly faster in the developing countries in 2015 and maintained a larger share of global biotech crops at 54% compared with only 46% for industrial countries (James, 2015).

Status of Transgenic Crops in India

The first transgenic plant experiment in the field was started in 1995 when *Brassica juncea* plant containing *Bar* gene regulated with plant-specific constitutive promoters and linked with *barnase* and *barstar* genes regulated with floral tissue-specific promoters were planted at Gurgaon (Haryana). Genetically, engineered insect-resistant cotton (Bt cotton) is the only crop approved in India for commercial cultivation (Choudhary, 2002). Currently, 16 genetic engineered crops containing the trait for drought and salinity tolerance, enhanced nutrient contents, insect resistance, increased shelf life, etc. are under various stages of regulatory pipeline in India (Giri & Tyagi, 2016).

India has emerged as one of the leading countries in the world in promoting local Research & Development in agricultural biotechnology in general and GM crops in particular. Further, it has by now been a fairly long experience in the functioning of comprehensive biotechnology and biosafety regimes that regulate the introduction and commercialization of GE crops and GE products. Both on its account (the second most populous country in the world with a broad industrial and technological base and a rapidly growing diversified economy) and as an important example to others, a critical analysis of the Indian effort and its outcome would be of considerable value

(Giri & Tyagi, 2016). Research activities in the non-GE categories of agricultural biotechnology and the commercialization of non-GE innovations in India date back to the early 1980s. Work on the GM categories began in the late 1980s. Although, over the last 15 years (1990–2004), many public sector research and development (R&D) institutions have been actively involved in developing a score of GM crops, with huge government support, none of these crops have yet reached the market (CAB, 2012; GEAC, 2004; Choudhary, Gheysen, Buysse, Meer, & Burssens, 2014).

India has the fourth largest area planted under GM crops, according to the International Service for the Acquisition of Agri-Biotech Applications (ISAAA, 2004–2008) (James, 2001). Farmers in India planted a total 11.6 mha under transgenics in 2014, behind the corresponding areas for Argentina (24.3 mha), Brazil (42.2 mha), and the United States (73.1 mha). The GM-crop acreage in India far surpassed China's 3.9 mha, while equaling that of Canada's 11.6 mha. ISAAA, a New York-based crop biotech advocacy group, has estimated the total global area under GM crops to have touched 181.5 mha last year, up from 175.2 mha in 2013. Significantly, the entire 11.57 mha GM-crop area in India last year consisted of Bt cotton. Nearly 96% of the country's cotton area is now covered by Bt hybrids. Bt technology has helped India to treble its cotton output from 13 million bales in 2002 (when it was introduced) to 40 million bales in 2014 (James, 2015; Singh et al., 2014).

ENVIRONMENTAL SAFETY AND POTENTIAL IMPACTS ON BIODIVERSITY

The potential risks to human and animal (livestock) health would arise from unexpected consequences of introducing new genes, such as the appearance of allergens, toxins, and carcinogens in GM-food and GM-livestock feed. Ecological and other environmental risks could arise from cross-pollination between GM crops and their indigenous wild relatives, potentially leading to loss of biodiversity, and the emergence and spread of pests, diseases, and weeds that could acquire the same resistances as are engineered into the GM crops. The socioeconomic safety of small farmers may be put at risk by the potentially negative impact on them of the agro-economic and trade consequences of GM crops (Scheffler, 1995; Gupta, Karihaloo, & Khetarpal, 2008).

The potential risks associated with genetically modified organisms (GMOs) have made it imperative for governments and civil societies to address the issue of “biosafety” in all the four major sectors of biotechnology: medical/pharmaceutical, agricultural, industrial, and environmental. In the context of GM crops, the concept of “biosafety” is, in principle, a broad one, covering three areas: the health safety of humans and livestock, the safety of the environment (i.e., ecology and

biodiversity), and socioeconomic safety (i.e., the economic and social impact on farmers, consumers, and different social classes, as well as on trade and economy in general). Although the biosafety regulations in force in industrialized countries [e.g., the United States, the European Union (EU), Canada, Australia, Japan] address only the health and environmental risks and exclude socioeconomic considerations, the regulations in some developing countries tend to include all the three areas (Ghosh, 1997; Choudhary et al., 2014). Countries have responded differently to the opportunities presented by GM crops and the risks associated with them. The composition of the “trade off” between potential benefits and risks in each case depends upon whether a government adopts a permissive, precautionary, or prohibitive policy approach to GM crops. For instance, the leading global exporters of grain, the United States, Canada, Argentina, and Australia adopted a permissive attitude very early on, with lower production costs and greater export profits presumably outweighing other considerations. But the EU, India, Japan, the Philippines, and others have taken a precautionary approach to the introduction, cultivation, and development of GM crops, whereas others like Ethiopia have, for the time being, decided against the introduction of GM crops altogether. The United States, Canada, and Australia would, however, argue that they too have very strict biosafety regulations, but they have other criteria than the “precautionary countries” that govern the balancing of benefits against risks which results in their relatively permissive approach.

The intense public debate both in developing and industrialized parts of the world on the introduction and commercialization of GM crops and GM products underlines the importance of mechanisms for the representation and participation of the public, in particular the civil society, in biosafety assessments, and decision-making. There ought to be active and effective channels of communication between the technology developers, the policymakers, and the wider public. Public confidence and trust are contingent on governments creating transparent and credible systems for the assessment risks and the enforcement of biosafety regulations that accord to international standards (UNEP-GEF, 2006).

The potential risks of biotechnology are manageable. For successful management, regulations have been constructed. The main areas of consideration for safety aspects in biotechnology are as below.

Gene Flow

The movement of genes between genomes of species or between environments is termed gene flow (Heinemann, 2007). However, from the biotechnology point of view, gene flow is the possibility that GM crops can hybridize with other related species and their wild relatives which leads to the transfer of the transgenes from the GM crops to their wild counterparts.

Engineered genes i.e., “transgenes” from GM crops might escape and be incorporated into wild populations (Healy, 2002) affecting the genetic consequences of advancing generations. Studies with transgenic herbicide-tolerant rapeseed (*Brassica napus*) in the United Kingdom showed that the gene flow rates through cross-pollination ranged between 0.0156% and 0.0038% at 200 and 400 m, respectively (Scheffler et al., 1995). On the one hand, agrobiotechnology has the potential to introduce the trait of interest, on the other hand, it also includes the risks of genetic movement of genes that otherwise would not exist in plants.

Threat to Super Weed

Most of the genetic transformation nowadays is being done for herbicide tolerance or insect resistance which is beneficial to protect our crops. However, gene flow due to cross-pollination for the traits involving resistance can result in development of tolerant/resistant weeds that are difficult to eradicate (Gupta et al., 2008). The development of resistance in organisms naturally is a long-term evolutionary process but incorporating resistance gene through cross-pollination among the compatible genomes could speed up this process considerably (Gupta et al., 2008). Transgenes might lead to the “super weed” evolution that confers a competitive benefit to the GM crop species’ wild relatives. As a result, our aim to develop crops resistant to specific chemical or herbicide or insecticide might disrupt the natural ecosystems. For example, if a transgene that confers pest or herbicide resistance is incorporated into a weedy relative of the GM crop, then the transgene would contribute to the evolution of increased weediness (Institute of Medicine and the National Research Council of the National Academies, 2004).

Threat to Destroy Genetic Diversity

A plant acquiring insect or disease-resistance genes will have more chances to become popular within a short time because of its enhanced fitness and preferential selection. This selection results in a shift in the original population structure (Soleri, Cleveland, & Cuevas, 2006). The selection would affect not only the concerned locus in which the wild-type alleles would be lost but also other loci that are closely linked to the fixed new allele (Gupta et al., 2008) leading to a final danger of “genetic erosion,” a situation where the affected gene becomes quite rare with severe chances of being disappeared from the natural genetic pool of the population (Singh et al., 2014). This reduced genetic diversity results in response to the GM organisms when farmers restrict themselves to a few popularly grown varieties/crops (Altieri, 2005).

Threat to Antibiotic Resistant

Horizontal gene transfer (HGT) is quite rare (Deni, Message, Chioccioli, & Tepfer, 2005), though documentation through evolutionary timescale gives evidences of HGT (Bergthorsson, Adams, Thomason, & Palmer, 2003). Though, there is possibility of transgenics that may have significant health and ecological impacts. Thus, attention for biosafety consideration is the possible consequences of HGT to the nontransgenic plants from the transgenic plants (Thomson, 2001; Celis et al., 2004). The possibility of HGT between bacteria and plants in either the soil or gut has been seen as a hazard associated with transgenic plants, particularly when this is related to the possible transfer of genes encoding antibiotic resistance. Thus, the level of significance of this concern as a risk depends on the likelihood of HGT and the magnitude of associated adverse outcome (Macdonald, 2012).

Health Concerns

The antibiotic-resistance-marker genes are generally used for the screening purposes of transgenic plants and organisms. But their effect on human health should also be ascertained beforehand to assure the critical level (Malik, 1999). In a press release, Gay and Gillespe (2005) stated that the contribution that recombinant bacteria might make is so small that antibiotic resistance markers do not pose a substantial risk to human health, because the contribution made toward antibiotic resistance by GM plants was quite negligible compared to the antibiotic prescription generally given in clinical and pharmaceutical practice (Islam & Miah, 2006). However, health benefits can also not be ignored, especially when we talk about reduced pesticide consumption in residual form in the food products. Some of the health benefits have been obtained in the form of biofortification i.e., augmented specific nutrients. One example is Golden rice with increased availability of vitamin A (Mannion & Morse, 2013). But Mannion and Morse (2013) have argued that still many gaps exist even at the cellular level because of the incomplete knowledge of how genes govern their expression. Transgenic soybean expressing allergenic seed storage protein from Brazil nut was reported to retain the allergenicity. The issue was so intense that it was withdrawn from release subsequently (Nordlee, Taylor, Townsend, Thomas, & Bush, 1996). Another example includes development of a genetically engineered pea expressing the “amylase inhibitor-1” protein from beans. It was also found to contain protein that could invoke an immune response (Prescott et al., 2005). Hence, no guarantees can be provided from scientists and plant researchers that a variety produced using GM process will be completely free from all the negative impacts on environment or human health. Therefore, the expression of the recombinant protein needs to be critically and carefully assessed by all means and from each aspect including the most important ones like health, allergenicity, toxicity, etc. (Ruibal Mendieta, Nagy, & Lints, 1997).

Impact on Nontarget Organisms

It is feared that the toxins produced by genetically engineered resistant plants may adversely influence nontarget insect species which either live foraging on the toxin-carrying plants or prey on insects that forage on such plants (Hilbeck, Baumgartner, Fried, & Bigler, 1998). Another concern on impacts of biotechnology is the probable harm of GM crops and their seeds to other, beneficial nontarget organisms (Kruft, 2001). A report on the harmful effects on the monarch butterfly of maize GE to express insecticidal endotoxins from the soil bacterium Bt caused much public interest. However, ecological studies conducted subsequently to evaluate the impact of pollen from GE crops to quantify the risks. The results showed that the large-scale commercial cultivation of Bt-maize hybrids did not pose a risk of significant effect to the monarch butterfly population. Further studies also demonstrated that Bt-expressing crops posed little risk to other nontarget insects, including beneficial insects like natural enemies and pollinators.

Trade Dilemmas

The conservation of agrobiodiversity is worldwide issue for the present era. Any threat associated with biodiversity needs to be handled with extra care because of the ease with which plant materials can cross national boundaries, the common example being air-borne pollen. In this regard, international agreements about the movement of plant materials are of high relevance to the regulation of agrobiotechnology for the nation (Richmond, 2006). Weak and faulty regulatory systems in the developing nations are the drawbacks that allow international agribusinesses and industries to promote GE technology without considering its impacts (Singh et al., 2014). Both the international as well as individual national governmental organizations need to function in a coordinated way so that the challenges for this aspect could be handled with an effective approach because it is an interdisciplinary issue spanning “trade, health, environment, intellectual property, and agriculture” (Gay & Gillespe, 2005). GM crops are not universally accepted throughout the international market. Some organizations such as EU have restricted the import of crops with inserted genes, citing concerns about the environment, ecology, and human health (Hamilton, 2001).

Consumer Acceptance and Regulatory Uncertainty

The safety assessment of GMOs is very extensive. It includes the evaluation of substantial differences between GM crops and their non-GM counterparts, molecular characterization, toxicity and allergenicity studies, and the assessment of the environmental impacts and unintended effects (Lusser, Parisi, Plan, & Rodriguez-Cerezo, 2011). There is reasonable evidence that

consumers are more comfortable with the use of genes from within the same species than transgenes originating from organisms such as bacteria (Schouten, Krens, & Jacobsen, 2006). Likewise, acceptance by the scientific community will depend on the classification under the GMO legislation. Crops obtained by the new plant-breeding techniques not yet commercialized and therefore their economic impact is not known. Therefore, the legal status of the new plant-breeding techniques will determine if they will be used only in specific projects or extensively by scientific community (Lusser et al., 2011).

Components Involved in Risk Analysis

Risk Assessment

Risk assessment of a biotech plant requires an understanding of the biology of the plant itself and the practices used in its cultivation. The risks involved or the criticizing concerns need to be carefully analyzed before making any use of biotechnological aspects so that the anticipated risks can be minimized. A prior estimation of the degree and rate of transgene escape will help to estimate the risk involved. No technology can make progress unless adopted by the target audience. Thus, adequate adoption measures to mitigating the amount of risks involved need to be analyzed. The kind of measures to be undertaken will be determined by the severity level to minimize the risk level. Through assessment should be conducted at grower, producer, processing, and marketing including adoption levels (Islam & Miah, 2006). Molecular breeders must ensure that the markers do not code for any toxin of considerable danger to consumer health, for which, it is essential to undertake assessment studies to determining gene flow (Malik, 1999).

This knowledge is important in identifying and evaluating potential environmental risks and also in designing any appropriate risk-management measures. These environmental safety concerns are not unique to plants produced using biotechnology and are also important when evaluating new varieties produced through conventional plant breeding. The objective of environmental safety assessment is to identify and evaluate any additional risks associated with the release and cultivation of these new plants in comparison with a conventional crop variety that has a history of safe use (DBT, 1990).

Most countries use similar environmental risk-assessment approaches, which include the following:

- Evaluating the role of the introduced gene in the plant and any changes in the plant's characteristics
- The possible unintended secondary effects on nontarget organisms
- The possibility that the modified plant could persist longer in the environment or invade new habitats
- The likelihood and consequences of the potential spread of newly introduced traits to related plants.

Risk Management

Evaristo de Jesus, Lanna, Vieira, Luiz de Abreu, and Ubeda de Lima (2006) defined risk management as “the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practice, and, if needed, selecting appropriate prevention and control options.” The framework principles of risk management should affirm the use of science-based safety assessments and management with the goals of protecting environment, ecology, human and animal health, while contributing to the prosperity and well-being of consumers. The approach should also guide one to create an enabling environment for biotechnology that strikes a balance between the necessary cautions in regulation while still allowing innovation to precede (Macdonald, 2012).

Risk Communication

Risk communication is also considered as part of the overall risk analysis. Risk communication is defined as “the interactive exchange of information and opinions throughout the risk analysis process concerning risks, risk related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management Decisions” (Evaristo de Jesus et al., 2006). Mayer has stated in his article, “it too late to keep the genie in its bottle” (Mayer, 1996). Research into GE will continue despite its bad or good impacts but still transgenics are being produced in enormously touching every kind of crop, food, drug, and industry. Such changes should have reliable communication regarding the sufficient information from researchers, policymakers, industries, and the government (Robinson, 1999). One of the strategies to be of effective use could be the increased participation from public in agricultural research and planning of programs from different perspectives in the future (Middendorf and Busch, 1997). The regulatory framework should communicate clear and transparent information requirements for the risk assessment to applicants and stakeholders. Clear communication of these requirements will enhance public confidence in the robustness of the risk assessment, assure that applicants have clear expectations, assure equal treatment for all applicants, and reduce delays delivering new technologies into the marketplace (Macdonald, 2012).

Regulatory Framework

The legislative measures being framed nowadays aim to safeguard the nation’s agricultural diversity and ecosystems resources from the unknown

consequences associated with genetically engineered crops. Strong enforcement mechanisms can improve implementation of these regulations by implementing a centralized regulatory authority and strengthening the complementary requirements to bridge the regulatory gaps (Richmond, 2006). In this regard, many biosafety guidelines to conduct research and apply biotechnology and GE have been evolved by the Asia-Pacific region. These regulations also take care of the transboundary movement of GE crops and their products. Harmonization of each and every regulation at the regional, national as well as international level to building capacities are critical for the coordinated implementation and generation of the benefits of biotechnology to farmers, researchers, and consumers (APCoAB, 2006). There are various organizations which vigilantly take account of the activities related to genetically engineered crops. For example, ICGEB (International Centre for Genetic Engineering and Biotechnology) focuses on training and research in biotechnology and molecular biology. It also ensures the developing countries to make safer use of biotechnological aspects. Likewise IFPRI (International Food Policy Research Institute) harmonizes research implications of GE technologies and policy for to alleviate poverty in the countries under development. CAMBIA (Centre for Application of Molecular Biology in International Agriculture) has been commissioned by most of the developing nations to develop a database aiming at indicating the technology ownership, an important issue determining whether scientists have “freedom to operate” in manipulation of certain crops and germplasm (Shrivastava, 2011). An information initiative of UNIDO (United Nations International Development Organization) named as BINAS (Biotechnology Information Network and Advisory Service) serves as a center for disseminating information of biotechnology laws and regulations. For the fulfillment of biosafety regulations in the concerned countries, the Global Environment Facility of the United Nations Environment Programme (UNEP-GEF) has supported these nations since 2001 to develop their own National Biosafety Frameworks (NBFs). NBF is a “combination of policy, legal, administrative, and technical instruments that are developed to ensure an adequate level of protection in the field of safe transfer, handling, and use of LMOs (living modified organisms) resulting from modern biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity taking into account risks to human health” (UNEP-GEF, 2006). The Food and Agriculture Organization of the United Nations (FAO) addresses requests for assistance from member governments for strengthening national biosafety systems, including thorough development and implementation of regulations, training of personnel of regulatory bodies in risk assessment and detection of GMOs, upgrading infrastructure, and improving communication and public participation in biosafety decision-making (UNEP-GEF, 2006). The important international regulatory institutions for biosafety regulation are also mentioned in Fig. 11.1 with their coverage of function and adopted countries.

Institution	Coverage	Active members (No. of countries)
Codex Alimentarius (Codex)	Food standards and levels	165
World Health Organization (WHO)	Pests and pathogens (plant)	191
International Plant Protection Convention (IPPC)	Health science and policy	107
International Epizootics Organisation (OIE)	Pests and pathogens (animal)	155
Food and Agricultural Organization (FAO) of the United Nations	Food security programmes	184
Cartagen Biosafety Protocol (BSP)	Transboundary movements of living modified organisms	130
Organization for Economic Cooperation and Development (OECD)	Hormonize science and/or progress	29
World Trade Organization (WTO)	Trade rules for all goods; dispute settlement mechanism	139
Regional Initiatives	Hormonize standard and policies	Various

FIGURE 11.1 Important international regulatory institutions for biosafety regulation.

To date, numerous protocols have been developed to deal with the regulatory issue of regulation at different levels. The Cartagena protocol on biosafety is the global treaty that reaffirms and incorporates the handy, safe, and precautionary approach to genetic manipulation and biotechnology. It promotes the uptake of GM technology and controlled adoption and has various provisions specifically addressing the safety concerns of consumers and the society. To enhance the benefit exploitation from the modern biotechnology while safeguarding users and consumers against potential risks, most of Asian as well as African countries have ratified and signed the Convention on Biological Diversity as well as the Cartagena Protocol on Biosafety (Francis, 2006). The continuing need for expert bodies is becoming highly important to account for the regulatory issues to authorities on genetic modification applications for approval of new planting material and genetically engineered foods. The basic need of such regulatory bodies is to include highly skilled experts and independent but effective decisions along with reliable acquaintance of means, laws, and authority to conduct vigorous analysis of any issue which according to them should be critically investigated.

Due to growing concern arising from GM crops throughout the world, the UNIDO/WHO/FAO/UNEP has built up an Informal Working Group on Biosafety. In 1991, this group prepared the “Voluntary Code of Conduct for the Release of Organisms into the Environment.” The ICGEB has also played an important role in issue related to biosafety and environmentally sustainable use of biotechnology. The biosafety rules are driven by multilayered decision-making structures.

BIOSAFETY REGULATORY SYSTEM IN INDIA

Over the past two decades, the advances made in modern agricultural biotechnology (agrobiotechnology) have opened up new frontiers in agricultural production. The new techniques for understanding and modifying the genetics of living organisms have led to large investments in agrobiotechnology R&D.

Since the late 1980s, the Government of India has given high priority and strong support to the development of agrobiotechnology. It is our belief that several interconnected ambitions are motivating the Indian authorities to provide this energetic backing: They want to make India one of the world's leading nations in agrobiotechnology and gear this indigenous technological development to Indian needs (Ahuja, 2005; DBT, 1990).

Most of this development has taken place in North America, Western Europe, and East Asia, with the United States being far ahead of the others. Today, six transnational agrochemical corporations (TNCs—Monsanto, Bayer, Syngenta, DuPont, Dow, and BASF) dominate the global arena for GM crops, from R&D to marketing (Gazette of India, 1989, 1993, 2010; GEAC, 2002).

In India, the first national biosafety rules were issued by the Ministry of Environment and Forests (MoEFs) as a notification and published in the Gazette of India, Extraordinary, Part II 3(i) on 5 December 1989. These 1989 rules are entitled “Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Micro Organisms or Cells.” They were followed in 1990 by department of biotechnology (DBT's) “Recombinant DNA Safety Guidelines,” which were then revised, expanded, and published in 1994 by DBT as the “Revised Guidelines for Safety in Biotechnology.” These three documents cover all the four main sectors of biotechnology: medical/pharmaceutical, agricultural, industrial, and environmental. Four years later, the agricultural biosafety guidelines were separated out, expanded, further revised, and published by DBT in August 1998 as the “Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant Parts” (GEAC, 2010, 2011a, 2011b).

The 1989 Rules, as well as the Guidelines of 1990, 1994, and 1998, have been made mandatory for all practitioners in India of genetic modification technologies under the Environment (Protection) Act of 1986. These four documents together constitute the national biosafety regulations of India. The 1998 Guidelines were amended in 1999 to clarify the mandates and roles of the RCGM (Review Committee on Genetic Manipulation) and the GEAC (Genetic Engineering Approval Committee) with respect to small-scale and large-scale field trials, and to establish the MEC (Monitoring and Evaluation Committee) (GEAC, 2011b; MOEF, 2011).

The concept of “biosafety” used in the regulations is a broad one. It covers the health safety of humans and livestock, environmental safety

(ecology and biodiversity), and economic impact. Health and environment safety aspects dominate the regulations, with economic impact given less prominence.

In addition to creating the RCGM and the GEAC at the central national level in New Delhi, and specifying their composition, the 1989 Rules also contain directives about the establishing and composition of biosafety committees at the institutional, state, and district levels. But, as will become clear later on in the present Section, the directive about creating state- and district-level biosafety committees has been ignored almost completely, with only three states having formally set up the state-level committees. District-level committees are entirely absent. As for MEC, its composition is laid down in the 1998 Regulations.

The UN-sponsored international Cartagena Biosafety Protocol to the Convention on Biological Diversity governing the transboundary (i.e., inter-country) movement of LMOs was agreed in Montreal in January 2000. It came into force in September 2003, having been ratified by the requisite number of countries. Although India has ratified the Cartagena Protocol, there is no information in the public domain to confirm that MoEF (which represents India in the CBD arena) or DBT has undertaken a formal and in-depth review of the relevant Indian national legislation to see whether and how the regulations need to be revised to accord to the provisions of the protocol (DBT, 1990; Choudhary et al., 2014).

The Structure and Functioning of the Regulatory Organization

In India, GM crops are regulated by the following three-tier structure (Choudhary et al., 2014):

- The *RCGM* under the Department of Biotechnology (DBT) of the Ministry of Science and Technology (MoST);
- The *GEAC* under the MoEF;
- The *Monitoring and Evaluation Committee (MEC)* under DBT/MoST; DBT provides the secretariat for RCGM and MEC, and the MoEF for GEAC.

Detailed work functions of the different regulatory committee are mentioned in Fig. 11.2 and in brief in the below text.

Recombinant DNA Advisory Committee

The Recombinant DNA Advisory Committee (RDAC) reviews developments in biotechnology at national and international levels and recommends suitable and appropriate safety regulations in India in recombinant research, their use, and applications. The RDAC is constituted by and based in the DBT (1990).

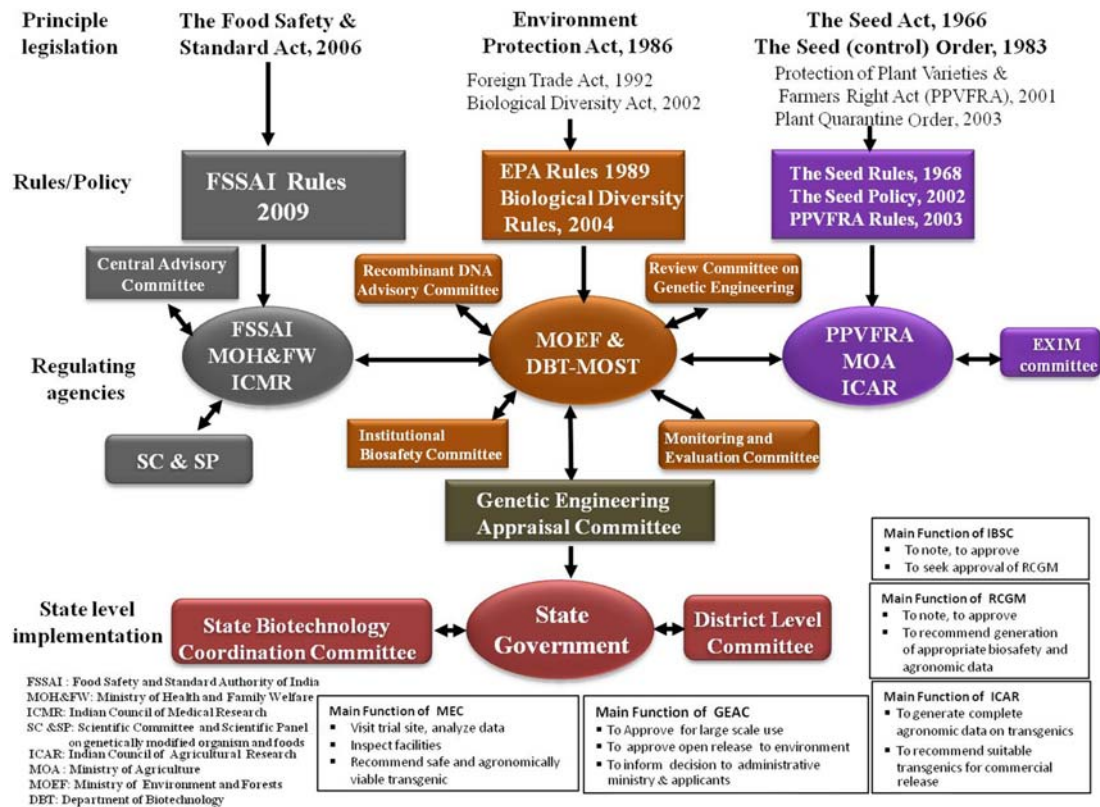


FIGURE 11.2 International coordinated regulatory framework on genetically modified crops in India. *Based on Choudhary et al. (2004).*

Institutional Biosafety Committee

The Institutional Biosafety Committee (IBSC) is constituted by the institution conducting research that handles microorganisms/genetically engineered organisms. The committee comprises the head of the institution involved in research, scientists engaged in DNA work, a medical expert, and a nominee of the DBT. The institutions involved in the process are required to prepare, with the assistance of the IBSC, an up-to-date on-site emergency plan according to the manuals/guidelines of the RCGM and make available copies to the District-Level Committee/State Biotechnology Coordination Committee and the GEAC ([Biosafety Issues Related to Transgenic Crops, 2004](#)).

Review Committee on Genetic Manipulation

The RCGM is also constituted by and based in the DBT. It monitors safety-related aspects of ongoing research projects and activities involving genetically engineered organisms. The Committee is entrusted with the responsibility of bringing out guidelines, specifying procedures and processes for activities involving genetically engineered organisms in research, use, and applications, all with the objective of ensuring environmental safety. All high-risk-category products, controlled field experiments, and containment conditions are reviewed by this committee which also lays down procedures for respecting or prohibiting production, sale, importation, and use of genetically engineered organisms or cells as listed in the schedule. Industries carrying out genetic research and projects come under the purview of the Committee.

Genetic Engineering Approval Committee

The GEAC is constituted and based in the MoEFs. It gives approvals for activities involving large-scale commercial use and release of hazardous microorganisms including imports of GMOs and recombinants in research and industrial production from the environmental angle. Where necessary, the Committee also restricts or prohibits production, sale, import, or use of GMOs.

State Biotechnology Coordination Committee

The State Biotechnology Coordination Committee (SBCC) is located at the state level constituted by the respective State Governments. It acts as the nodal agency at the state level to assess damages, if any, from the release of GMOs. It has the powers to inspect, investigate, and take punitive action in case of violations of statutory provisions through the Nodal Department and the State Pollution Control Board/Directorate of Health/Medical Services. The Committee is also required to periodically review the safety and control

measures in various industries and institutions handling genetically engineered organisms or hazardous microorganisms and take on-site control measures.

District-Level Committees

The District-Level Biotechnology Committee (DLC) is constituted below the State Government level in the district where biotechnology projects function. It is headed by the District Collector (who is the chief executive of the Government at this level of administration) and monitors safety regulations in installations engaged in the use of GMOs and hazardous substances. The Committee investigates compliance with r-DNA guidelines and reports violations to the SBCC or the GEAC. The Committee also coordinates activities with a view to meeting emergency situations arising from accidental releases.

Monitoring and Evaluation Committee

This committee is required to undertake field visits at experiment sites, suggest remedial measures to adjust original trial design, assist the RCGM in collecting and analyzing field data, and collect or cause to collect information on comparative agronomic advantages of transgenic plants. The structure of the biosafety decision-making structure in India is depicted in [Fig. 11.1](#). As [Fig. 11.2](#) illustrates, there are four domains involved in the life cycle of a biotech product that is based on GMOs. These are the prerelease, research, release, and postrelease domains. A product runs through the four domains, which are characterized by the presence of the six structures described above. The RDAC is in the prerelease domain as it triggers research through its initial approval mechanisms. The RCGM functions in the research domain, closely monitoring the process of research and experimental releases. Commercial releases of organisms or biotech products containing GMOs come under the purview of the GEAC, a body that dominates the release domain. The Monitoring and Evaluation Committee and the SBCC and DLC basically occupy the postrelease domain, although they contribute to the research domain activities through data-provisioning to the RCGM. The IBSC undertakes monitoring and implementation of safeguards at the R&D sites, under the close supervision of the RCGM, the SBCC, and the DLC.

BOTTLENECK OF BIOSAFETY ISSUES AND FEEDBACK OF BIOSAFETY REGULATORY AUTHORITIES

The private companies are subject to the same regulations about institutional biosafety committees (IBCs) as the public sector institutions. Larger companies have, in principle, the scientific staff to constitute competent IBCs and the infrastructure and resources to comply with the legally obligatory

biosafety procedures and measures at all stages of the development of GM crops. But it is debatable whether the same is true of the small companies, whose main concern would be to get the backcrossing and testing work done as inexpensively as possible, which means, among other things, keeping investment in in-house scientific expertise and infrastructure to a bare minimum. Generally, small companies seeking the assistance of some public sector state agricultural universities to get their biosafety testing done.

It is all too likely that the composition and mode of operation of the IBCs of the larger companies are not exempt from the constraints and shortcomings that affect the IBCs of public sector institutions. The companies have neither a policy nor a practice of consultations with GM-concerned nongovernmental and civil society organizations and publicists, whether in planning, deciding, designing, and conducting their GM activities. The same is true of the public sector institutions. Both groups tend to regard the GM-concerned nongovernmental organizations (NGOs), central services organizations (CSOs), and publicists with hostility. They are angry about the campaigns and activism of these organizations.

Some companies elicited the following remarks, views, and attitudes about the current biosafety regulatory regime in India. Similar concerns also emerged in our interviews with selected public sector R&D institutions which are as follows:

- The biosafety guidelines and regulations are very cumbersome, stringent, and time consuming. A literal compliance would be too difficult to achieve.
- Of particular concern are the problems posed by the requirement to test for potential toxic effects on livestock of feed and forage derived from GM crops (e.g., seeds from ginning GM cotton, postharvest residues, and GM-forage maize), not least due to the difficulties in obtaining permission by the relevant authorities to conduct the tests and the impact of the campaigns by animal rights activists 26.
- The activism of the anti-GM nongovernmental and civil society organizations is a “nuisance.”
- The transgenes that have been cleared by the regulatory authorities as being safe in the context of a given crop (e.g., the Bt-genes in Bt-cotton) should be “deregulated” and be exempt henceforth from the regulations, when used in other crops, i.e., the case-by-case approach should be interpreted as applying to transgenes and “transgenic events” and not to individual crops.
- The regulations should be made less stringent for GM crops whose transgenes are derived from “closely related plant species.”
- The smaller and less well-endowed companies want access to an increased number of public sector risk-assessment facilities (e.g., national laboratories and agricultural universities) spread over country.

- The replacement of the present regulatory system (with its dispersed, unclear and confusing mandates, responsibilities, and powers) by a new, single, integrated authority with a comprehensive mandate and a wide range of responsibilities, with the power to implement the regulatory regime with speed and efficiency.
- A historically and traditionally conditioned atmosphere of mutual distrust still bedevils relations between government authorities and the private sector.

Pressure Points for the Biosafety Regulations

There have been instances where requests have been rejected by the GEAC. These include the release of Mech 915 Bt cotton hybrid and the import of a corn–soy blend. Despite this trend of approvals and rejections, the functioning of biosafety regulations have been subjected to criticism both by industry and civil society groups. Although industry associations consider these regulations as affecting their growth, civil society groups consider biosafety regulations as not being strong enough to check the introduction of potentially harmful biotechnology products. As a result, the regulations have undergone changes. Some of these changes were effected to allay industry apprehensions, whereas in some cases, they were brought in to address civil society concerns.

Civil Society

The civil society proposals, which are based on [Sahai \(2004\)](#), highlight the following:

- Regulatory processes are not tight enough, do not rely on precautionary principles and give in easily to new biotech products of dubious nature.
- An independent advisory body comprising representatives of scientific disciplines, adivasis (tribals), panchayat-raj institutions (local self-government), and the legal profession may be constituted.
- A statutory body may be set up to conduct environment assessment, facilitate risk management and risk communication so as to foster decision-making about the safety of a GM crop from an environmental, human, and animal health perspective. It is further suggested that the same body may be entrusted with the responsibility of postrelease monitoring.
- Data on field trials should be made available to the public, which needs to be involved in decision-making. Annual review reports on GM products also need to be submitted to the Parliament.
- Greater attention may be paid to the views of the SBCC and DLCs, prior to giving approval for GM crops.

Industry

The proposals mooted by the industry, as contained in Suresh (2004), stress on the following:

- The present decision-making apparatus is dilatory and elongated. A case in point is the time lag in the release of the Monsanto–Mahyco Bt cotton case where a period of 7 years elapsed before the final approval for commercial release was obtained from the regulatory authorities.
- The GEAC gets into matters which are not purely biosafety concerns. Thus, the insistence of the GEAC on evaluating the economic performance of Bt cotton and its decision to hold an inquiry into Shanta Biotech's clinical trial data for the company's recombinant Streptokinase drug are quoted as instances of the body dabbling with issues other than core biosafety concerns.
- The Association of Biotechnology Led Enterprises (ABLE) has demanded the introduction of a modified procedure that reduces the existing multilayers of biosafety decision-making. The association has suggested removal of the GEAC from its apex status and elimination of its independent role in approving human clinical trials data for r-DNA medicines. ABLE wants the GEAC to approve commercial release of r-DNA drugs on the basis of documentation submitted by the RDAC and the RCGM for environmental clearance.

Ministry of Environment and Forests

As pointed out earlier, the GEAC has already shed its powers of approval for research-related experimental releases to the RCGM, following the amendments to the biotechnology safety guidelines made in 1999. The present proposal is for further reducing the significance of the GEAC as the apex decision-making body in the biosafety regulatory system.

- Concedes that there are delays and problems in the approval of GM products;
- The IBSC may be eliminated, and the collection, analysis, and submission of greenhouse data may be made by the research unit directly to the RCGM;
- The MEC currently submits reports directly to GEAC, for all the three stages being, large fields trials, commercial release based on data collected by the Indian Council for Agricultural Research (ICAR), and postrelease monitoring. The GEAC, in turn, provides the monitoring data to the RCGM. The MoEF wants the GEAC to shed commercial release and postrelease monitoring functions to the Department of Agriculture and the ICAR, respectively.

Crosscutting Issues

Crosscutting issues are those that are common to each of the five preceding elements, and they are often the most challenging factors to address and resolve. They are, however, the issues that will ultimately dictate the scope of a national policy on biosafety, and the conversion of policy into practice. Crosscutting issues affect the implementation of the system designed to assess biosafety, and perhaps more importantly, those nontechnical factors that are crucial to public acceptance and confidence in the decisions that are made by government on behalf of the people.

The twin issues of public information and participation have to do with the degree of transparency in a regulatory system, and the degree to which the public has input either into the formulation of regulatory policy or into specific regulatory decisions. Transparency refers to the extent to which governments provide information on why and how certain products are regulated, how risk assessments are performed and decisions made, and as well, the conclusions and decisions that have been reached. Transparency can also involve the perceived independence and objectivity of the regulatory decision-makers.

Human, financial, and infrastructure resources largely determine the scientific and administrative capacity of any country; they obviously influence any biosafety-related policy or program. Funds must be available to develop and implement a national biosafety system to support the infrastructure required, such as buildings, labs, equipment, and computers; to facilitate communication and public participation; to train scientific and regulatory personnel; and to foster the research required to assure that risk assessments are sound.

CONCLUSION

Evidences clearly reveal that, the acreage of transgenic crops is increasing day by day and will play a major role in coming in Biosafety regulation. Regulatory mechanisms for the release of transgenics are in place. A Plant Variety Protection Bill (PVP) has been cleared by the Indian Parliament and will soon become operational. These developments should encourage industry to invest in the seed sector. The Government of India and the Department of Biotechnology are committed to fund research in the area of crop biotechnology. The support from the Government for funding research in crop biotechnology has increased. If tangible products come through, given the importance of agriculture to the economy, prospects of higher financial outlays for crop biotechnology are bright.

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Chapter 12

Genetic Engineering and Public Perception

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INTRODUCTION

Biotechnology is considered as one of the main economic development forces of the 21st century; it presents equally far-reaching legal, moral, and ethical implications for the society. Gene technology—a controversial and emotive subject—is central to the application of biotechnological techniques in many industries. Genetic engineering (GE) specifically is one type of genetic modification that involves an intended targeted change in a plant, microbe, or animal gene sequence, which affects a specific result. Typically, it is a directed manipulation of the hereditary material based on Recombinant DNA technology that consists of the splicing of a piece of DNA to a suitable carrier which is then introduced in a convenient cell that allows the “passenger” or “foreign” DNA to express itself in the new surroundings (Fig. 12.1).

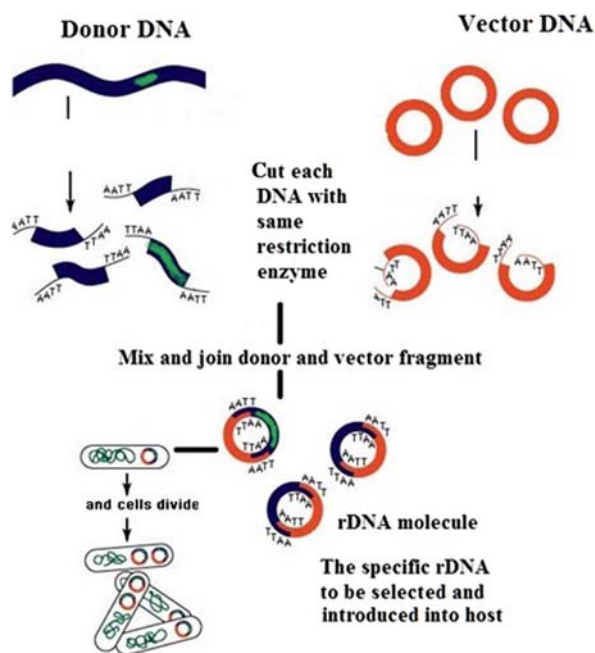


FIGURE 12.1 Recombinant DNA technology.

GE differs from conventional breeding by allowing genes to be transferred across taxonomic boundaries. With GE, genes can be transferred not only between closely related species (e.g., when a gene coding for a disease resistance is transferred from wheat to rice) but also between completely different species (e.g., when a gene coding for cold tolerance is transferred from fish to strawberry plant). Organisms genetically modified (GM) by rDNA technology are referred to as transgenic plants, animals, or microbes as the case may be. This may be done to express a gene that is not native of the plant, animal, and microbe or to modify an endogenous gene.

GE has been particularly effective in incorporating herbicide resistance in canola and soybeans as well as insect resistance in maize and cotton (FAO, 2004). Genetically engineered farm animals can be created to enhance food quality. For example, pigs have been genetically engineered to express the D12 fatty acid desaturase gene (from spinach) for higher levels of omega-3, and goats have been genetically engineered to express human lysozyme in their milk. GE can also be utilized to create designer companion animals e.g., major cat allergen Fel d 1 is a small and sticky protein that is secreted by the cats' sebaceous glands (Reininger et al., 2007). Some companies like Felix Pets create hypoallergenic cats by using GE techniques to remove the gene coding for the allergen Fel d 1 (<http://felixpets.com/>). Companion species have also been derived by cloning. The first cloned cat "CC" was

created in 2002 (Shin et al., 2002), and after just a few years, scientists created the first cloned dog, “Snuppy” (Lee et al., 2005). It remains feasible that genetically engineered pets could become part of day-to-day life for practicing veterinarians, and there is evidence that clients have started to enquire about GE services, in particular the cloning of deceased pets (West, 2006). Alterations in microbes are also frequently utilized. Genetic alteration in microbes is mainly focused on the nature of the products; however, their release in environment may be hazardous. In human beings, two types of cloning have been studied for human welfare—reproductive cloning and research cloning.

As GE allows gene transfer across taxonomic boundaries, some people consider GMOs to be unnatural organisms that violate the laws of nature (Bruhn, 1992). Others consider this distinction arbitrary, countering that most foods consumed today have been radically modified overtime through mutations or deliberate selection (Bruhn, 1992).

Due to ethical reasons, human gene manipulation has been either opposed or supported according to needs. Sentiments among opponents of reproductive cloning are more strongly felt and less likely to change than are those among supporters. Only a small portion of the opposition to cloning is based on perceptions of its physical danger. More often, people cite beliefs that it is “morally wrong”, “interferes with human distinctiveness and individuality”, “could be used for questionable purposes like breeding a superior race”, or conflicts with their religious beliefs.

In the industrialized world, public policymakers on biotechnology have been influenced by the concerted interests of governments, industries, academia, and environmental groups. Public perception is an important variable in this equation which is often difficult to balance. Public perception may be defined as “a representation of public consciousness or will” (Savigny, 2002) and is a collective generalization of individual perspectives. Globally, such policies are being developed within a climate of tension and conflicting aims with the central theme being—should regulations be dependent on the characteristics of the products modified by recombinant DNA technology or on the use of the technology per se? The product-versus-process debate has continued for many years and exposes conflicting views on what should represent public policies on new technology development.

Agriculture, animal, microbe-based industries, and clinical interventions in human beings in terms of gene replacement therapy are loosely included under the global term biotechnology. But application of GE in plants, animals, and microbes utilizes characteristically unique ancillary techniques, and of course, cater to different objectives. As we are discussing a wide array of organisms, an equally diverse set of issues and objections is to be expected. Hence, these areas will be treated under separate headings in the following pages.

ANIMAL GENETIC ENGINEERING

GE animals are those animals that have a change in their nuclear or mitochondrial DNA achieved through a deliberate human technological intervention. This may be accomplished by addition, deletion, or substitution of some part of the genetic material or insertion of foreign DNA. Those animals that have undergone induced mutations (e.g., by chemicals or radiation—as distinct from spontaneous mutations that naturally occur in populations) and cloned animals are also considered to be genetically engineered due to the direct intervention and planning involved in creation of these animals.

GE technology has numerous applications involving companion, wild, and farm animals, and animal models used in scientific research, though many such animals are still in the research phase, there are a variety of intended applications for their use.

Applications

Companion Animals

By inserting genes from sea anemone and jellyfish, zebrafish have been genetically engineered to express fluorescent proteins—hence, the commonly used term “GloFish.” GloFish began to be marketed in the United States in 2003 as ornamental pet fish; however, their sale sparked controversial ethical debates in California—the only US state to prohibit the sale of GloFish as pets (West, 2006). In addition, to the insertion of foreign genes, gene knock-out techniques are also being used to create designer pets, e.g., hypoallergenic cats where the gene that codes for the major cat allergen Fel d 1 has been knocked out (<http://felixpets.com/>). Companion species have also been derived by cloning e.g., “cat CC” and “dog Snuppy” (Shin et al., 2002; Lee et al., 2005).

Wild Animals

The primary application of GE to wild species involves cloning. This technology could be applied to either extinct or endangered species; for example, there have been plans to clone the extinct thylacine and the woolly mammoth (West, 2006). Holt, Pickard, and Prather (2004) point out, “As many conservationists are still suspicious of reproductive technologies, it is unlikely that cloning techniques would be easily accepted. Individuals involved in field conservation often harbour suspicions that hi-tech approaches, backed by high profile publicity would divert funding away from their own efforts.” However, cloning may prove to be an important tool to be used alongside other forms of assisted reproduction to help retain genetic diversity in small populations of endangered species.

Farm Animals

As reviewed by [Laible \(2009\)](#), there is “an assorted range of agricultural livestock applications (for GE) aimed at improving animal productivity; food quality and disease resistance; and environmental sustainability.” Productivity of farm animal species can be increased using GE. Examples include transgenic pigs and sheep that have been genetically altered to express higher levels of growth hormone ([Laible, 2009](#)). Such advances may add to the nutritional value of animal-based products. Farm species may be genetically engineered to create disease-resistant animals ([Laible, 2009](#)). Specific examples include conferring immunity to offspring via antibody expression in the milk of the mother, disruption of the virus entry mechanism (which is applicable to diseases such as pseudorabies), resistance to prion diseases, parasite control (especially in sheep), and mastitis resistance (particularly in cattle).

GE has also been applied with the aim of reducing agricultural pollution. The best known example is the Enviropig: a pig that is genetically engineered to produce an enzyme that breaks down dietary phosphorus (phytase), thus limiting the amount of phosphorus released in its manure ([Laible, 2009](#)). There has been resistance to the commercialization of GE animals for food production, primarily due to lack of support from the public ([Gaskell, Allum, & Stares, 2003](#)). However, this may change. A recent debate over genetically engineered AquAdvantage Atlantic salmon ([Table 12.1](#)) may result in these animals being produced commercially ([Assessment Report of FDA, 2010](#)). Effort has also been made to generate genetically engineered farm species such as cows, goats, and sheep that express medically important proteins in their milk. According to [Dyck, Lacroix, Pothier, and Sirard \(2003\)](#), “transgenic animal bioreactors represent a powerful tool to address the growing need for therapeutic recombinant proteins.” In 2006, ATryn became the first therapeutic protein produced by genetically engineered animals to be approved by the Food and Drug Administration (FDA) of the United States. This product is used as a prophylactic treatment for patients that have hereditary antithrombin deficiency and are undergoing surgical procedures.

Research Animals

Biomedical applications of GE animals are numerous and include understanding of gene function, modeling of human disease to either understand disease mechanisms or aid drug development and xenotransplantation. Through the addition, removal or alteration of genes, scientists can pinpoint what a gene does by observing the biological systems that are affected. Although some genetic alterations have no obvious effect, others may produce different phenotypes that can be used by researchers to understand the function of the affected genes. GE has enabled the creation of human-disease

TABLE 12.1 Scientific and Societal Concern About Genetically Modified Salmon

Area of Concern	Societal Acceptance	Societal Rejection	Communication of Uncertainty
Human health and food safety	Cheaper food; increased food security; omega-3 source	Unnaturalness; less acceptance for food use than medical and pharmaceutical applications	Incomplete data; human health risk not yet identified
Animal health and welfare	Fish are associated with less concern than mammals	Animal welfare concerns emerging lower down the evolutionary chain	No data on unhealthy transgenic fish; these will be the focus of societal concern
Environmental safety	Contained farms with no contact with external environment	Lack of evidence to support 100% sterility if escape occurs; deliberate release	Uncertainty regarding no impact following environmental release of animals; Trojan gene and wild population extinction

models that were previously unavailable. Animal models of human disease are valuable resources for understanding how and why a particular disease develops, and what can be done to halt or reverse the process. As a result, efforts have focused on developing new genetically engineered animal models of conditions such as Alzheimer’s disease, amyotrophic lateral sclerosis (ALS), Parkinson’s disease, and cancer. However, these models do not always accurately reflect the human condition, and care must be taken to understand the limitation of such models (Wells, 2010). The use of genetically engineered animals has also become routine within the pharmaceutical industry, for drug discovery, drug development, and risk assessment. According to Rudmann and Durham (1999), transgenic and knock-out mouse models are extremely useful in drug discovery, especially when defining potential therapeutic targets for modifying immune and inflammatory responses. Specific areas for which GE animal models may be useful are screening for drug-induced immunotoxicity, genotoxicity, and carcinogenicity, as well as understanding toxicity-related drug-metabolizing enzyme systems. Perhaps, the most controversial use of genetically engineered animals in science is to develop the basic research on xenotransplantation—that is, the transplant of cells, tissues, or whole organs from animal donors into human recipients. In relation to organ transplants, scientists have developed a genetically engineered pig with the aim of reducing rejection of pig organs

by human recipients (Logan & Sharma, 1999). This particular application of GE is currently at the basic research stage, but it shows great promise in alleviating the long-waiting lists for organ transplants, as the number of people needing transplants currently far outweighs the number of donated organs. However, as a direct result of public consultation, a moratorium is currently in place preventing pig-organ transplantation from entering a clinical trial phase until the public is assured that the potential disease transfer from pigs to humans can be satisfactorily managed (Einsiedel & Ross, 2002). No clinical trial involving xenotransplantation has yet been approved (Table 12.2).

Ethical Issues and Public Perception

The GE of animals has increased significantly in recent years, and the use of this technology brings with it ethical issues, some of which relate to animal welfare—defined by the World Organization for Animal Health as “the state of the animal and how an animal is coping with the conditions in which it lives” (OIE—Terrestrial Animal Health Code, 2011). These issues need to be considered by all stakeholders, including veterinarians, to ensure that all parties are aware of the ethical issues at stake and can make a valid contribution to the current debate regarding the creation and use of genetically engineered animals. As a result of the extra challenges that genetically engineered animals bring, governing bodies have started to develop relevant policies, often calling for increased vigilance and monitoring of potential animal welfare impacts (MacArthur, Potter, & Harding, 2006). Some key ethical issues identified in the case of genetically engineered animals used in science are described in the following sections.

Invasiveness of Procedures

The generation of a new genetically engineered line of animals often involves the sacrifice of some animals and surgical procedures (e.g., vasectomy, surgical embryo transfer) on others. These procedures are not unique to genetically engineered animals, but they are typically required for their production. During the creation of new genetically engineered animals (particularly mammalian species), oocyte- and blastocyst-donor females may be induced to superovulate via intraperitoneal or subcutaneous injection of hormones; genetically engineered embryos may be surgically implanted to female recipients; males may be surgically vasectomized under general anesthesia and then used to induce pseudopregnancy in female embryo recipients. In addition, all offsprings need to be genotyped, which is typically performed by taking tissue samples, sometimes using tail biopsies or ear notching (Robinson et al., 2003).

TABLE 12.2 Applications of Genetically Modified Animals

Application	Rationale	Example on the Market	Acceptance/Societal Responses	
Xenotransplantation	Therapeutic—cells, tissues, and organs e.g., human to pig	Expected in next few years	Medical	+ve
			Ethical and religious issues	−ve
Bioreactors	GM animals producing therapeutics in their milk or eggs	Atryn goat	Medical	+ve
		Rhucin or ruconest	Ethical and religious issues	−ve
			Environmental escape	−ve
Animal productivity	Increased growth, disease resistance or product quality	Nonlicensed	Food	−ve
		Aquabounty salmon	Marketing	−ve
		Environ pig	Species dependent	+ve (fish > mammals)
Companion animals	Hyperallergenic cats faster race horses	GloFish	Consumer data Equity issues	−ve
Disease models	Rodents, rabbits and pigs used to model human diseases	Most common form of GM animals (mainly rodents)	Medical	+ve
	Test therapeutics		Ethical and religious issues	−ve
			3Rs	−ve

Large Number of Animals Required

Many of the embryos that undergo GE procedures do not survive, and of those that do survive, only a small proportion (between 1% and 30%) carries the genetic alteration of interest (Robinson et al., 2003). This means that large numbers of animals are produced to obtain genetically engineered animals that are of scientific value, and this contradicts efforts to minimize animal use. In addition, the advancement of GE technologies in recent years has led to a rapid increase in the number and varieties of genetically engineered animals, particularly mice (Ormandy, Schuppli, & Weary, 2009). Although the technology is continually being refined, current GE techniques remain relatively inefficient, with many surplus animals being exposed to harmful procedures.

Unanticipated Welfare Concerns

Little data has been collected on the net welfare impacts to genetically engineered animals or to those animals required for their creation, and GE techniques have been described as both unpredictable and inefficient (Robinson et al., 2003). The latter is due, in part, to the limitations in controlling the integration site of foreign DNA, which is inherent in some techniques (such as pronuclear microinjection). In such cases, scientists may generate several independent lines of genetically engineered animals that differ only in the integration site (Verbeek, 1997), thereby further increasing the numbers of animals involved. With other more refined techniques that allow greater control of DNA integration (e.g., gene targeting), unexpected outcomes are attributed to the unpredictable interaction of the introduced DNA with host genes. These interactions also vary with the genetic background of the animals. Such variations have been frequently observed in GE mice (Yoshiki & Moriwaki, 2006). Interfering with the genome by inserting or removing fragments of DNA may result in alteration of the animal's normal genetic homeostasis, which can be manifested in the behavior and well-being of the animals in unpredictable ways. For example, many of the early transgenic livestock studies produced animals with a range of unexpected side effects including lameness, susceptibility to stress, and reduced fertility (Laible, 2009). A significant limitation of current cloning technology is the prospect that cloned offspring may suffer some degree of abnormality. Studies have revealed that cloned mammals may suffer from developmental abnormalities, including extended gestation, large birth weight, inadequate placental formation, and histological effects in organs and tissues (e.g., kidneys, brain, cardiovascular system, and muscle). One annotated review highlights 11 different original research articles that documented the production of cloned animals with abnormalities occurring in the developing embryo and suffering of the newborn animal and the surrogate mother (Weaver & Morris, 2005).

Genetically engineered animals, even those with the same gene manipulation, can exhibit a variety of phenotypes; some causing no welfare issues and some causing negative welfare impacts. It is often difficult to predict the effect of a particular genetic modification on an individual animal, so genetically engineered animals must be monitored closely to mitigate any unanticipated welfare concerns as they arise. For newly created genetically engineered animals, the level of monitoring needs to be greater than that for regular animals due to the lack of predictability. Once a genetically engineered animal line is established and the welfare concerns are known, it may be possible to reduce the levels of monitoring if the animals are not exhibiting a phenotype that has negative welfare impacts.

To aid this monitoring process, some authors have called for the implementation of a genetically engineered animal passport that accompanies an individual animal and alerts animal care staff to the particular welfare needs of that animal (Wells et al., 2006). This passport document is also important if the intention is to breed from the genetically engineered animal in question, so the appropriate care and husbandry can be in place for the offspring.

With progress in GE techniques, new methods (Miller et al., 2007; Yang et al., 2009) may substantially reduce the unpredictability of the location of gene insertion. As a result, GE procedures may become less of a welfare concern over time.

Ethical issues, including concerns for animal welfare, can arise at all stages in the generation and life span of an individual genetically engineered animal. An accepted “Three Rs” doctrine (Russell & Burch, 1959), ethics of animals have raised awareness on the welfare of animals used in research, as it promoted the “Refinement” of research techniques in order to minimize animal suffering and distress, “Reduction” in the number of animals used, and “Replacement” of these animals where possible so as to avoid the use of animals in research. However, despite the steps taken to minimize pain and distress, there is evidence of public concerns that go beyond the Three Rs and animal welfare regarding the creation and use of genetically engineered animals (Macnaghten, 2004). The Brambell Report (1965) was highly influential in this matter, as it identified the “five freedoms” an animal should be recognized: freedom from hunger and thirst, from discomfort, from pain, injury or disease, from fear and distress, and freedom to express natural behavior (Kaiser, 2005). Issues of animal welfare have gradually been voiced in the European political arena and elsewhere since the mid-1970s and have been major issues in the European research policy since circa 1986, when the European Convention and Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes were adopted.

In India, the Indian National Science Academy (INSA) has developed guidelines for use of animals in scientific research. Considering the knowledge generated internationally over the years and the guidelines of WHO, NIH-associated NRC, the United States and European Union, the INSA

guidelines have been updated. The Prevention of Cruelty to Animal Act of 1960 has provided the constitution of a committee under the Animal Welfare Board to control and supervise experiments on animals. It has also provided for inspection of all animal holdings through designated inspectors, and the institutions not abiding by the standard requirements can be prosecuted.

All scientists working with laboratory animals must have a deep ethical consideration for the animals they are dealing with. From the ethical point of view, it is important that such considerations are taken care of at the individual, institutional, and finally at the national level.

Individually, each investigator has an obligation to abide by all the ethical guidelines laid down in this regard at institutional level. The head of the institution maintaining animals for scientific experiments should constitute an Animal Ethics Committee for experimentation to ensure that all experiments conducted on animals are rational, do not cause undue pain or suffering to the animals, and only minimum number of animals are used. The constitution and terms of reference of the Animal Ethics Committee should be well defined.

An Animal Ethics Committee should include: a senior biological scientist of the institute, two scientists from different biological disciplines, a veterinarian involved in care of animals, the scientist in-charge of animal facility, a scientist from outside the institute, a nonscientific socially aware member, and a member or nominee of appropriate regulatory authority of Government of India. A specialist may be coopted while reviewing special projects using hazardous agents such as radioactive substance and deadly microorganisms.

The Animal Ethics Committee has to examine all projects involving the use of animals before implementation, to ensure that minimum number of animals is used in the project and the ethical guidelines are strictly adhered to. It will also examine that the scientists and technicians handling animals possess adequate skill to perform the experiment. All animals will be maintained under standard living conditions, and experiments will be conducted with care. All invasive experiments will be conducted under proper anesthesia and on termination of an experiment; the animal will be humanely sacrificed under anesthesia. Before disposal, it must be ensured that the animal is clinically dead.

Ethical Guidelines for Use of Animals in Scientific Research

1. Animal experiments should be undertaken only after due consideration of their relevance for human or animal health and the advancement of knowledge.
2. The animals selected for an experiment should be of an appropriate species and quality, and minimum number should be used to obtain scientifically and statistically valid results.

3. Investigators and other personnel should treat animals with kindness and should take proper care by avoiding or minimizing discomfort, distress, or pain.
4. Investigators should assume that all procedures which would cause pain in human beings may cause pain in other vertebrate species also (although more needs to be known about the perception of pain in animals).
5. Procedures that may cause more than momentary pain or distress should be performed with appropriate sedation, analgesia, or anesthesia in accordance with accepted veterinary practice. Surgical or other painful procedures should not be performed on unanesthetized animals.
6. At the end, or when appropriate during an experiment, the animal that would otherwise suffer severe or chronic pain, distress, discomfort, or disablement that cannot be relieved or repaired should be painlessly euthanized.
7. The best possible living conditions should be provided to animals used for research purpose. Normally, the care of animals should be under the supervision of a veterinarian or a person having adequate experience in laboratory animal care.
8. It is the responsibility of the investigator to ensure that personnel conducting the experiment on animals possess appropriate qualifications or experience for conducting the required procedures. Adequate opportunities have to be provided by the institution for in-service training of the scientific and technical staff in this respect.
9. In vitro systems to replace or reduce the number of animals should be used wherever possible. These systems could be living (tissue/organ culture, lower animals, and microorganisms, etc.) or nonliving systems (chemicals, mechanical models, mathematical models, computer simulation, etc.).

Other Ethical Issues

GE also brings with it concerns over intellectual property and patenting of created animals and/or the techniques used to create them. Preserving intellectual property can breed a culture of confidentiality within the scientific community, which in turn limits data and animal sharing. Such limits to data and animal sharing may create situations in which there is unnecessary duplication of genetically engineered animal lines (Ormandy, 2010).

Methods of disposal of euthanized GE animals are restricted irrespective of their application. The reason for this is to restrict the entry of genetically engineered animal carcasses into the natural ecosystem until the long-term effects, and risks are better understood. Environment Canada (<http://www.ec.gc.ca/>) and Health Canada (<http://www.hc-sc.gc.ca/>) offer specific guidelines in this regard.

It will become increasingly important for veterinarians to inform themselves about any special care and management required by genetically engineered animals. As animal health professionals, veterinarians can also make important contributions to policy discussions related to the oversight of GE as it is applied to animals and to regulatory proceedings for the commercial use of genetically engineered animals.

Regarding cloned animals, many advocacy groups have argued, from scientific reports, that among the few cloned animals that survive a cloning process, many are deformed or have significant abnormalities. As scientists have stated, a low-success rate and abnormalities are, for the present time, inherent to cloning techniques. But technical improvement is entirely conceivable. Animal welfare associations have been raising such issues in countries like the United Kingdom and India. Religious sentiments could be one major reason why animal activism in the latter has found firm roots, whereas in the West, it may be because of the writings of some secular philosophers.

HUMAN GENETIC ENGINEERING

It may have been in the realm of science fiction for a long time, but GE has now entered the realm of human possibility. On the one hand, the term brings to mind hopes of futuristic therapy and fantastic human enhancement; yet on the other, it generates fears of a world where eugenics is common, and the human genome and values are corrupted.

Applications

Mitochondrial DNA transfer and somatic cell nuclear transfer (SCNT) are the two currently employed methods to create embryonic stem cells (ESCs). Mitochondrial DNA transfer has been promoted as a way for people with mitochondrial disease to have healthy children with their own nuclear DNA, and SCNT-produced stem cells have the potential to lead to innovative therapies that will treat various diseases and injuries.

Mitochondrial DNA Transfer

Mitochondria are an essential component of a functioning cell. Their primary function is to provide 90% of the energy requirements of the human body. They are also crucial to the ability of a cell to form new DNA and RNA molecules. Mitochondria are produced from special “mitochondrial DNA” (mtDNA) that is separate from the primary nuclear DNA. Only a small portion of the genome is mtDNA, less than 1%, which are only 37 genes (out of a total 20,000–25,000 in the human genome).

Mitochondrial DNA is passed directly from the mother to the offspring; the father’s mitochondrial DNA does not because the mitochondria of the sperm do not fuse with the ovum during fertilization. Disorders of this DNA

may cause malfunctions throughout the body, including stunted growth, depressed immunity, diabetes, cardiac, renal or hepatic disease, visual and auditory deficits, muscle weakness, and loss of coordination among other neurological problems including seizures. The effects are generally visible before the age of 10 and may be fatal in some cases.

Mutations in mitochondrial DNA are seen 10 times more often than in nuclear DNA. Approximately 1 in 10,000 people suffers from some form of mitochondrial disease today, and as many as 200 people per 10,000 are carriers. There is no known cure at present, so a woman suffering from or carrying mitochondrial disease cannot reproduce without passing on her disease to her children as well unless a donated egg is used.

Mitochondrial DNA transfer offers such women a chance of having their own, healthy biological children. In this technique, fertilized nuclear DNA from the patient's egg is inserted into a donor egg whose nucleus has been removed. Thus, a child with the genetic traits of the parents and only the mitochondria of the donor is born.

Stem Cells Research

The prospect of using stem cells for cell-based therapy, called regenerative medicine, has long been a medical dream. Stem cells are unspecialized cells that can differentiate into a multitude of different cell types. They can fall into two categories: **ESCs**, found in the developing embryo and **somatic (adult) stem cells**, which act as a repair mechanism for specific tissues. ESCs have much greater longevity. Scientists hope to be able to use them in the future to create new organs, repair tissue damage, and treat illnesses. Studying the mechanism through which these cells replicate and specialize may also lead to a greater understanding of diseases like cancer and Alzheimer's. In addition, stem cells could be used to test experimental drugs.

Due to the ethical issues surrounding the harvesting of stem cells from embryos, a technique called cellular reprogramming was developed in 2007. In simple terms, a somatic cell is made to revert to an unspecialized, stem cell-like state, producing an "induced pluripotent stem cell" or iPSC. These, however, are not identical to ESCs; researchers are still investigating exactly how they differ. Another technique SCNT and adult stem cells to create ESCs are in trial research. This involves removing the DNA from an unfertilized egg and replacing it with a somatic cell's. The unfertilized egg then begins to divide after it is exposed to various chemicals and an electric pulse. Eventually, a blastocyst is created (the size of approximately 150 cells), and ESCs can be obtained. The cells gleaned are genetically identical to the individual from whom the original somatic cell was derived. This is a similar technique to the one that created the famous cloned sheep Dolly in 1996.

Ethical Issues and Public Perceptions

It is vital to engage a public and transparent discussion and debate about the potential benefits and risks of these GE techniques now, before the first attempted application. We must proceed cautiously and in full appreciation of the risks involved. The use of mitochondrial DNA transfer is currently banned in the United Kingdom, though in March 2013, the Human Fertilisation and Embryology Authority officially recommended that the practice be allowed, and the issue is currently under review.

With new technology comes a new ethical question. Are guidelines good enough? Is there a need to have a law that will regulate this burgeoning field of research? The field of human GE is particularly rife with ethical concerns, because it involves tampering with some of the basic mechanisms of human biology. Perhaps, the most common concern is the untested nature of mitochondrial DNA transfer technology. Before the FDA ban on the technology in the United States in 2001, there was a slightly unofficial experimental trial at the Institute for Reproductive Medicine and Science of St. Barnabas in New Jersey, where ooplasmic transfer was used to produce approximately 15 human babies. Whether all these babies were entirely healthy, however, remains unclear, and no follow-up experiments have been completed to verify the results ([Tachibana et al., 2009](#)). Two different groups have used the technology, apparently successfully, in rhesus monkeys, one in 2009 and another in 2010. The offspring produced appeared to be healthy, with no complications ([Tachibana et al., 2009](#)). However, some scientists and ethicists fear in the absence of methods to predict how the technology will work in humans, proceeding with the research would be in effect using human babies as lab experiments, hence, unethical. They opine that the parents' desire to give their genes to their offspring cannot outweigh the potential damage to their future offspring especially when the safer options of adoption and egg donation are available.

Besides, though people may accept GE when it affects only the current individual, most view it as unethical to modify genetic material that will be passed on to future generations in effect altering whole genetic lines of human beings. They say that future individuals have the right to an unmodified human genome. In addition, in the face of such a new untested technology, it would be wise to monitor such cases lifelong. The question that arises here is about the efficacy of follow-up studies which would necessarily be voluntary. Another issue is the fact that the unborn child has no say in whether he or she wishes to participate in this experimental procedure and work with the accompanying complications. Furthermore, some scientists argue that there is evidence to support that mitochondrial genome also influences the identity of a person.

SCNT technology also comes with similar ethical problems. All regulations which prohibit ESC research are opposed to nuclear transfer techniques

for similar reasons including the commoditization of the embryo. Within countries where deriving stem cells from supernumerary embryos are permitted, only some national regulations have authorized the use of nuclear transfer techniques to obtain human ESCs. In France, despite support from a part of the researchers, it has not been authorized by the Biomedical Agency. It is explicitly authorized and regulated in countries such as the United Kingdom, Sweden, Canada, the United States (some states e.g., California), Japan, China, and India, whereas it has not been forbidden in Finland. Interestingly, many national ethics committees produced reports quite favorable to human nuclear transfer, years before the law became more flexible, e.g. France, Germany, Finland, Sweden, and the United Kingdom. In 2006, the Indian Council of Medical Research formulated some far-reaching guidelines on biomedical research. In 2007, another set of guidelines that regulate stem cell research were issued, this specifically bans cloning of humans. The most common concern is centered on SCNT's connection to cloning. As the SCNT technique is the same technology that scientists used to famously clone the sheep Dolly in 1996, there is concern that SCNT could be used to clone a human as well.

Many people believe it is unethical to create embryos and then destroy them in order to harvest the stem cells. Some people are of the opinion that embryos are human beings and as such have the full moral rights of an individual. However, Mitalipov, a pioneer of SCNT, says that embryos not "fertilized naturally," and most likely without the potential to grow into humans should not be viewed as true people. An additional issue is the ethics of egg donation. In light of the health risks to egg donors, women may be exploited to donate eggs for financial compensation. Women from unprivileged backgrounds may participate in egg donation without a true appreciation for the risks involved, leading to a lack of informed consent.

Social Implications

In addition to the ethical issues discussed above, there is a social perspective to these techniques which cannot be overlooked. Mitochondrial DNA transfer would produce a child with DNA from three sources instead of the usual two. Apart from parental nuclear DNA, it should possess donor mitochondrial DNA. Questions have been raised as to if and how this mitochondrial DNA would affect the child's parental situation. Would the child essentially have three parents? Would he or she need to have a relationship with the donor? Would the donor have any parental rights? Or would it simply be like donating an organ—which, some scientists have argued, results in the transference of more DNA than mitochondrial DNA transfer does. The answers to these questions could have important impacts on society.

PLANT GENETIC ENGINEERING

Plants with favorable characteristics had been produced from thousands of years by conventional breeding methods. However, modification to produce desired traits in plants used for food began about 10,000 years ago. Desirable traits are selected, combined, and propagated by repeated sexual crossings over numerous generations. These changes, along with natural evolutionary changes, have resulted in common food species that are now genetically different from their ancestors. Advantageous outcomes of these genetic modifications include increased food production, reliability, and yields; enhanced taste and nutritional value; and decreased losses due to various biotic and abiotic stresses, such as fungal and bacterial pathogens. These objectives continue to motivate modern breeders and food scientists, who have designed newer genetic modification methods for identifying, selecting, and analyzing individual organisms that possess genetically enhanced features. This is a long process taking up to 15 years to produce new varieties. GE not only allows this process to be dramatically accelerated in a highly targeted manner by introducing a small number of genes, but can also overcome the barrier of sexual incompatibility between plant species and vastly increase the size of the available gene pools.

With a span of 5 years agricultural biotechnology and the development of GM, foods have progressed from small-scale field trial to large commercial plantings worldwide (Table 12.3). According to recent figure, some 5.5 million farmers are presently growing crops obtained through agricultural biotechnology that cover more than 58 million ha. With regards to the developing countries, the technology offers the opportunities to complement traditional techniques and improves their agricultural systems.

A large number of GM crops and foods have been developing to address hunger and malnutrition. These include maize and cotton cultivars modified with the *Bacillus thuringiensis* gene for insect resistance (Bawa & Anilakumar, 2013; FAO, 2008), herbicide-tolerant Canola and soybean (Rowe, 2004), and golden rice that has increased vitamin A content (Bonny, 2003). However, persistent controversy and claims that these products may not be perfect and may be harmful to humanity, and the environment has created considerable concern.

Although advances in modification methods hold the potential for reducing the time, it takes to bring new foods to the marketplace, an important benefit of a long evaluation period is that it provides opportunities for greater assurance that deleterious features will be identified, and potentially harmful new varieties can be eliminated before commercial release. It is both prudent and preferable to identify potentially hazardous products before they are made commercially available, and with few exceptions, standard plant-breeding practices have been very successful in doing so.

TABLE 12.3 Transgenic Agricultural Research

Transgenic Crop	Gene Inserted	Desired Trait	Institutions Involved
Bt maize	Cry II gene from <i>Bacillus thuringiensis</i>	Insect resistance	KARI/CIMMYT financed by Syngenta
Potato	CP-PVX (coat protein from potato virus X)	Viral resistance	KARI/Monsanto
FlavrSavr	Antisense of ACC (1-aminocyclopropane 1-carboxylic acid) deaminase enzyme from	To prevent production of ethylene	Calgen
	Antisense of PG enzyme (polygalacturonase enzyme)	To prevent degradation of pectin	
Tobacco	Bacterial chitinase from <i>Serratia marcescens</i>	Fungal resistance	Central Research Laboratories, Hokko Chemical Ind. Co.
Rice	NHX gene from <i>Arabidopsis thaliana</i> encoding antiporter	Resistance to abiotic stress (tolerance to salinity)	Metahelix Life Sciences, Bangalore
Soybean	<i>aroA</i> gene and <i>gox</i> gene	Herbicide resistance	Monsanto co.

Ethical Issues and Public Perceptions

GE is one of the most powerful 21st century technologies, and its use is driving the new “green revolution” in agriculture. In global terms, the use of GM crops has increased rapidly and steadily since the first commercialization in the United States in 1996. During the 17 years of commercialization, the global area of GM-crop production has increased 100-fold, from 1.7 million ha in 1996 to 170.3 million ha in 2012, comprising more than 12% of the world’s arable land (James, 2012a,b). This increase was driven by benefits such as use of more benign herbicides and significantly reduced pesticide use and, as a result, decreased environmental impact associated with application of chemicals to these crops (Brookes & Barfoot, 2013). Although there are great potential benefits from the use of GM crops (Brookes & Barfoot, 2013), the potential risks to human health and the environment have been the subject of concern and debate (Kikuchi, Watanabe, Tanaka, & Kamada,

2008; Li, Romeis, Wu, & Peng, 2014; Romeis et al., 2013; Wolt et al., 2010). A biological hazard that is envisaged concerns the release of genetically engineered strains of agriculturally important species. The development of a paragon superproducer may lead to the exclusive use of this particular strain. Such a development would result in the loss of several other naturally occurring strains.

It was assumed that an individual's attitude toward agricultural biotechnology, in general and GM foods in particular, is determined by his or her perceptions of risks and benefits of this technology. The perceived risks arise from the uncertainty about the safety of GM foods as well as the potential negative social and environmental effects of GE. The benefits may include potential nutritional, economic, and environmental benefits (e.g., reduced pesticide use to grow crops). An individual's acceptance of GE will ultimately depend on his or her perception of the net benefit i.e., the difference between the benefits and risks of this technology.

Global Trends in Public Perceptions of Genetically Engineered Crops and Foods

Responses to public opinion studies are very sensitive to the conditions surrounding the surveys. The precise wording of questions, the methods used to select respondents and administer the survey and the type of background information provided to the respondent can influence the results substantially (Hoban, 2002). Today, subsistence farmers in Kenya eke out meager livings and the ability to provide enough food for survival is often less than assured and the vital importance of staple crops such as maize, sweet potatoes, and cassava cannot be overstated. For example, research has shown that the term biotechnology is much more accepted by the lay public than the term genetically modified organism (GMO) (Hoban, 2002). Although differences in terminology can cause the level of acceptance to differ by 10%–20%, many surveys used terms such as GE, Genetic Modification, and GMOs besides biotechnology interchangeably, accounting for an unknown part of differences observed across countries and over time. Similarly, sample size and methods used to select and administer the surveys can influence the results (ESA, 2004). Most surveys reviewed involved approximately 1000 interviews per country representing a confidence level of just over 3% (ESA, 2004).

The most extensive international study of consumer attitudes toward GMOs was conducted by Environics International (2000). In this study, more than 35,000 respondents from 35 countries were asked whether they agreed that the benefits of biotechnology outweighed the risks (Hoban, 2002).

National differences in public support for GM food lies greatly in these risk assessments, since, for instance, European citizens, and Canadian citizens, consider GM food much more risky and less beneficial than US citizens do (Gaskell et al., 2004, 2006). Biosafety concerns for the release of

GMOs in the environment have been a major issue in the European public mind, whereas, by contrast, they are of little concern to Asian consumers from China, Indonesia, and the Philippines. Over two-third of respondents in the United States, Colombia, Cuba, Dominican Republic, China, India, Indonesia, and Thailand agreed that the benefits of GM crops are greater than the risks. Fewer than 40% of consumers agreed to this statement in France, Greece, Italy, Spain, and Japan (Hoban, 2004). Japan and South Korea were more negative than other parts of the world. The United States leads industrialized countries in support of GMOs. Overall, people in the developing countries tend to be quite supportive of GM crops (ESA, 2004).

In India and Japan, opposition from NGOs gained importance at the turn of the century. “Ethical” arguments were rapidly presented, from the idea that GMOs are not natural, to more precise issues concerning the safety of their release into the environment. Some associations, such as Greenpeace, also voiced the economic and social issue that a massive development of GM crops could lead to increasingly powerful biotechnology companies taking control of agriculture at the farmers’ expense. Institutions and researchers, including the US Department of Agriculture, answered to the so-called unnaturalness of GMOs through the media, insisting that such definitions ignored history, as common fruits and vegetables all have been voluntarily genetically altered—thus, highlighting continuity between traditional and modern biotechnologies.

The idea that GM technologies might help feed hungry people (humanitarian argument) is often put across. The “humanitarian” argument is not new, yet it has become more influential as scientific progress seems to go in this direction, working on drought-resistant, climate-specific, or vitamin-supplemented GMOs for instance. The media has given voice to the idea that “the Developing World Simply Can’t afford to do Without Agricultural Biotechnology” (Maralla & Bharathi, 2015) and that GM crops could alleviate hunger or malnutrition. In Asia, against such ethical arguments, NGOs such as Greenpeace, the Third World Network, and the Research Foundation for Science, Technology and Natural Resource Policy in India have argued that the real issue was not shortage of production, but the poors’ incapacity to have access to existing food (Nuffield Council on Bioethics, 2003).

Associations have also stressed that Gene Use Restriction Technologies (GuRTs)—coined “Terminator” technologies by the Canadian group RAFI (now the ETC Group)—aiming to create sterile plants would deny farmers their ancient right to save and exchange seeds from previous harvests (The Corner House UK, 1999). This has led to more classical economic arguments such as corporate-control-threatening farm livelihoods of the very poor. The “Terminator” argument has been very influential in countries such as India, where monopoly on a living organism is seen as unacceptable and where seeds saving, exchange and replanting are identified as farmers’ rights (De Castro, Sy, Alvarez, Mendez, and Rasco, 2004). Although GuRT techniques

were still in the research phase, international NGOs together with farmer associations and lobbies such as Karnataka State Farmers' Association (KRRS) used these as their main argument against any GMO development. Public opposition to Bt cotton in India, led to voluntary declarations from industry not to use GuRT there, and to the government eventually refusing applications for open-field Bt cotton agriculture in 2001 (Ramanna, 2006). Such involvement from farmers and their representatives, which was observed throughout South Asia (De Castro, Sy, Alvarez, Mendez, and Rasco, 2004) is, however, two-sided. In India, the government's decision to finally approve commercial release of Bt cotton in March 2002, indeed, is a result of pressure from Indian farmers themselves, who concluded their first alliance with industry (Ramanna, 2006). The Kisan Coordination Committee, Liberty Institute, Confederation of Indian Industry and Federation of Farmer's Associations of Andhra Pradesh claimed and managed to obtain recognition of the right for farmers to choose what they considered the most efficient seeds against recurrent pest attacks (Ramanna, 2006). Since then, confronted with farmers calling for freedom in agricultural choices, the national influence of international NGOs has been less important and innovative in India, as solid networks between industry and farmers associations have come into place.

In many countries such as India, respect for animal welfare is rooted in religious beliefs. However, "Animals and birds are thought not only as Vahanas or Vehicles on which God rides, but much more useful as well. Over the centuries this has brought about a very healthy respect in the Indian mind for all forms of life. The cow is sacred not because it is a divine vehicle alone, but because it has an overall utility value. Buddhism and Jainism carry this attitude further, leading to vegetarianism and respects for all living beings. To the Sufis, steeped in equally considerate attitudes the prevalent Indian mind set was extremely acceptable. Thus, in the East, regardless of specific sects or religions, the attitude to other life forms was not exploitative, but appreciative. Even pigs, boars, buffaloes and monkeys are referred in holy books and the Indian mind set can become easily sensitive when it comes to these animals. These religious sentiments could be one major reason why the animal activism in this country has found firm roots, while in the West it may be because of the writings of some secular philosophers" (Indian Council of Medical Research, 2000).

Indian Trends in Public Perceptions of Genetically Engineered Crops and Foods

The Government of India (Allocation of Business) Rules 1961 assigned the responsibilities of "biodiversity conservation" and "environment protection" to Ministry of Environment and Forests (MOEF) in 1961 (Government of India, 1961). Thereafter, MOEF began regulating GMOs and products thereof under the existing Environmental Protection Act 1986, commonly referred as EPA

1986, which was enacted by the Parliament of India in 1986. Although the EPA 1986 does not describe GMOs and GM crops in the law per se, it lays down the legislative provisions to regulate “hazardous substances” (Hazardous substance of the EPA 1986 section 3, 2 (iv) means any substance or preparation which, by reason of its chemical or physicochemical properties or handling, is liable to cause harm to human beings other living creatures, plants, microorganism, property or the environment) and to make administrative rules to regulate environmental pollution caused by hazardous substances. GM crops, GMOs, and the products of GE were de facto categorized as “inherently harmful” in the same manner as hazardous substances that cause harm to human beings or other living creatures, property, or the environment. Notably, the EPA Rules 1989 to regulate GMOs and GM crops were issued by an “administrative order” through publication in the Gazette of India vide notification GSR 1037(E) dated December 5, 1989 and came into force vide notification S.O.677(E) dated September 13, 1993 ([Gazette of India, 1989, 1993](#)). The Rules apply to hazardous microorganisms that are pathogenic to human beings, animals, or plants, regardless whether they are GM. The Rules 1989 not only regulate research, development, and large-scale commercialization of GM crops but also order compliance of the safeguard through regulatory approach, postapproval monitoring of violation, and noncompliance ([Ahuja, 2005](#)). The Rules define competent authorities and composition of such authorities for handling of various aspects of GMOs. ([Fig. 12.2](#) describes the interministerial coordinated regulatory framework on GM crops in India.)

The EPA Rules 1989 were made central to the biosafety regulation of GM crops, whereas others applied to food safety and quality of seeds for sale and connected matters ([Asia Law House, 2005](#)). These guidelines were revised in 1994 as “Revised Guidelines for Safety of Biotechnology.” Realizing the need for comprehensive guidelines for transgenic plants in the mid-1990s, DBT framed and released a comprehensive guide for GM crops in 1998 referred to as “Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants, and Plant Parts” to regulate GM crops and products. These guidelines have also been revised time to time, till date ([Randhawa & Chhabra, 2009](#)).

The regulatory system evolved along with the import of transgenic crops, GM mustard by Proagro and Bt cotton by Mahyco for RandD purpose in the mid-1990s. The development of GM mustard *Brassica juncea* was discontinued in 2001 by Bayer CropScience that acquired Proagro at the penultimate stage of commercial approval. The insect-resistant Bt cotton varieties primarily *Gossypium hirsutum* developed by Mahyco in collaboration with Monsanto received approval for commercial cultivation in 2002. The approval process witnessed an intense debate and protest as a result of the evolving nature of regulatory system responding to scientific, technological, policy, and social challenges. After gaining a considerable field-level experience with BG-I Bt cotton event, GEAC approved three new cotton

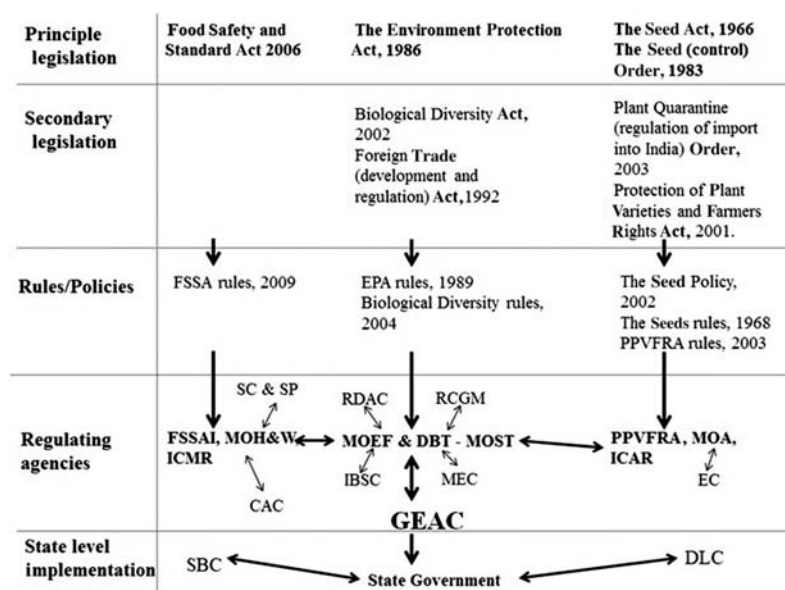


FIGURE 12.2 The interministerial coordinated regulatory framework on GM crops in India. RDAC, Recombinant DNA Advisory Committee; GEAC, Genetic Engineering Appraisal Committee; RCGM, Review Committee on Genetic Manipulation; MEC, Monitoring and Evaluation Committee; IBSC, Institutional Biosafety Committee; SBCC, State Biotechnology Coordination Committee; DLC, District Level Committee; ICMR, Indian Council of Medical Research; FSSAI, Food Safety and Standard Authority of India; SC & SP, Scientific Committee and Scientific Panel on Genetically Modified Organisms and Foods; MOEF, Ministry of Environment and Forest; DBT, Department of Biotechnology; MOA, Ministry of Agriculture; ICAR, Indian Council of Agricultural Research; PPVFRA, Protection of Plant Variety and Farmer's Right Authority; MoH&FW, Ministry of Health and Family Health Welfare; MOST, Ministry of Science and Technology; EC, EXIM Committee; CAC, Central Advisory Committee.

events in 2006 namely BG-II Bt cotton expressing Cry1Ac and Cry2Ab developed by Mahyco, Event-1 Bt cotton expressing Cry1Ac developed by JK Seeds, and GFM event expressing Cry1Ab and Cry1A developed by Nath Seeds. Subsequently, two more events namely BNLA-601 expressing Cry1Ac developed by UAS, Dharwad, and MLS-9124 expressing Cry1c developed by Metahelix Life Sciences in 2008 and 2009, respectively. In the meantime, the regulatory agencies processed the hybrid-based regulatory approval of Bt cotton, a more cumbersome and time-consuming process to the event-based approval mechanism in 2009 (MOEF, 2009). This system allowed regulators to closely evaluate, monitor, and assess risk and benefits of other GM crops including Bt brinjal, Bt/HT maize, Bt/HT cotton, Bt cauliflower, Bt rice, and GM mustard that were extensively field tested in the country. The GEAC in the 97th meeting held on October 14, 2009 concluded that Bt brinjal event EE-1 is safe for environmental release. However,

GEAC referred the decision to approve or reject the environmental release of Bt brinjal to MOEF (GEAC, 2009a). Bt brinjal event EE-1 was developed indigenously by Mahyco in collaboration with the University of Agricultural Sciences, Dharwad, the Tamil Nadu Agricultural University, Coimbatore, and the Indian Institute of Vegetable Research, Varanasi. The project was subjected to a rigorous and stringent regulatory approval process strictly complying with two dozen regulatory permits issued by RCGM and GEAC between 2000 and 2009. On farm-level conditions, Bt brinjal demonstrated an effective resistance to the deadly fruit and shoot borer *Leucinodes orbonalis* that required sprays twice a week resulting in 15–40 insecticide sprays or more in one season and caused significant losses of up to 60%–70% in commercial plantings (GEAC, 2009b; Choudhary & Gaur, 2009). In spite of that, MOEF decided to impose a moratorium on the commercial release of Bt brinjal on February 9, 2010 preempting long-term health risk and liability relating to loss of biodiversity (MOEF, 2010).

According to the EPA Rules 1989, GEAC is the statutory committee with a sole mandate to regulate GM crops and accords approval for contained, confined, and environmental release of GM crops in the country. GEAC is also entrusted with various provisions of the EPA Rules 1989 that require approval including the following:

1. Approval for the import of GM microorganism for research purpose [section 7(1)].
2. Approval for the use of hazardous microorganisms and recombinants in research and industrial production [section 4(3) (i)].
3. Approval for all kind of experimental field trials of GM crops [section 4 (3)(i)].
4. Approval for measure concerning discharge of hazardous microorganisms [section 7(3)].
5. Approval for licenses for scaling up pilot project involving genetically engineered microorganism [section 7(4)].
6. Approval for the deliberate release of genetically engineered organism [section 9(2)].
7. Approval for certain substances containing genetically engineered organisms (section 10).
8. Approval for food stuffs containing GMOs (section 11), and finally,
9. Hold power to revoke any approval [section 13(2)].

To facilitate regulatory decision-making and guarantee the safety of GM events before commercialization, biosafety regulation from laboratory research to approval for use of a novel GM plant event is divided into five stages, namely: (1) laboratory research, (2) pilot testing, during which small-scale biosafety tests are conducted within a contained system or under controlled condition, (3) environmental release field testing, during which medium-scale biosafety tests are conducted under natural conditions

with appropriate safety control measures, (4) preproduction testing, during which large-scale biosafety tests are conducted prior to application for a GMO biosafety certificate, and (5) application for biosafety certificates [Office of Agricultural Genetic Engineering Biosafety Administration (OGEBA), 2010].

The outcomes of introducing insect-resistant Bt cotton in Indian agriculture have been exceptional and unparalleled. The adoption and impact of Bt cotton not only contributed to doubling national cotton production but also delivered broad based, inclusive, and scale-neutral benefits to smallholder cotton farmers (James, 2012a,b; Kathage & Qaim, 2012; Subramanian & Qaim, 2009). Cotton farmers irrespective of their farm size quickly replaced the commonly used chemical-based crop protection method, which carries great health risks of workers, with the insect-resistant Bt cotton—a more efficient and cost-effective method of crop protection. In a short period of 10 years, around 7 million small cotton farmers representing >90% of total cotton farmers in the country adopted Bt cotton on 9.5 million hectares, occupying 88% of the cotton crop in 2011–12 (James, 2012a,b; PIB, 2012; RajyaSabha, 2012a). A series of studies confirmed a significant reduction in the number of insecticide sprays by half (Kranthi, 2012). As a result, the sale of insecticides and spraying equipments has plummeted in rural India. The use of chemical insecticides on cotton measured in active ingredient, halved from 46% of total insecticides used in agriculture in 2001–02 to 20% in 2011–12 despite a significant increase in cotton cultivation area in the same period (Kranthi, 2012). Likewise, studies reported that 9 of 10 farmers who planted Bt cotton repeated planting in subsequent years—a very high level of repeat adoption in documented trend, the allegation of failure and ill effects of Bt cotton continues to rage. Without presenting empirical evidences, Bt cotton has been alleged to cause sheep and cattle deaths, adversely affect human health and to some extent attributed to have caused farmers' suicides in Vidharbha region of Maharashtra. "There seems to be no evidence of direct relationship between Bt cotton and farmers' suicides," stated in the Rajya Sabha of the Parliament of India (RajyaSabha, 2012b). Similarly, several empirical assessments of Bt cotton have found no evidence of a resurgence of farmers' suicides in the country (Guillaume & Sengupta, 2011; IFPRI, 2008). Notably, Bt cotton has been referred as neither suicide seeds nor silver bullets, but a remarkably valuable technology (Herring, 2013).

On the contrary, at national level, the average cotton lint per hectare yield had increased significantly in the 13-year period, 2002 to 2014; and impressively, India contributed one quarter of this global total (Choudhary & Gaur, 2015). Farmers preferred to grow Bt cotton because it became comparatively more profitable than other crops such as millets and legumes in nontraditional cotton areas of semiarid tropics.

Golden Rice and other GMOs exist in many spheres—physically in farmers’ fields and grocery stores, symbolically in pressing current issues, and rhetorically in online information centers and public debate. GMOs are considered useful in public debates as mentioned by corporate regarding world hunger, human rights, moral boundaries, validity of scientific research, government and international regulations. It is interesting that public opinion of GMOs is generally considered polarized. People are usually characterized as completely pro- or anti-GMO and rarely as conditionally pro- or anti-GMO depending on the context.

These stereotypes are created by powerful interest groups that control the information available to the public. Both pro- and anti-GMO interest groups contend that “crop genetic modification should be judged and rejected as a whole rather than analyzed as the varied enterprise that it is” (Stone, 2002). Biotech advocates, in an evocative narrative of hope, herald GM, high-yield crop varieties as the solution to world hunger (Glover, 2010; Schurman & William, 2010). Others argue that hunger is caused by systemic disproportionate access to resources, a problem that won’t be solved by increased food production (Zerbe, 2004).

Two news reports about Golden Rice as a window into public opinion of GMOs, noting the arguments made in the comments and the sources cited for support. Golden Rice is a variety of rice GM to produce beta-carotene, the precursor of vitamin A. It is called “golden” rice because the modification also causes it to turn yellow. Golden Rice was developed as a humanitarian effort by nonprofit groups as a way to combat vitamin A deficiency (VAD) in the third-world countries where rice is staple crop. It would be free for farmers who make less than US\$10,000 a year. This makes it a very interesting case because most GMOs are developed by corporations and sold for a profit, one of the main critiques voiced by anti-GMO activists. Although public opinion of Golden Rice is tied up in perceptions of the private biotechnology industry, it is a case that forces people to consider and debate corporate control, patents, and farmer’s rights separate from the safety, environmental effects, and cultural implications of the technology itself. This allowed a spectrum of opinions to emerge from the comments. Golden Rice is arguably a “propoor” technology being used to help people without profit or benefit to the developers. Because of this, however, corporations like Monsanto use it to advocate for all GMOs, even those that generate profit (Stone, 2002). Some have even dubbed it the “poster child” for industrial biotechnology (Stone, 2002). The promotional use of Golden Rice by the industry is intended to obscure differences between the sectors of biotechnology as much as it is to draw attention to this one invention, reinforcing a monolithic and positive image of GM crops (Stone, 2002).

Some anti-GMO activists are calling Golden Rice a “Trojan Horse,” that will allow the evil biotech industry entry to the third-world countries (Stone, 2002). On the other hand, flat out rejection of all GMOs based on a dislike

of private corporate practice is arguably inconsiderate of welfare in the third-world countries where a publically funded technology could save millions from VAD (Stone, 2002; Charles, 2013).

All the information and opinion about GMOs can be found on the Internet but readers have to be careful about which sources you trust. Interested institutions, governments, companies, and individuals may take this information to frame to support their position.

Monsanto (the largest multinational biotech corporation) has become the symbol of everything people dislike about industrial agriculture. Other important topics are also spun in the GMO debate including corporate control of the regulatory process, lack of transparency for consumers, lack of choice for farmers, ever-increasing use of pesticides on ever-expanding monocultures; and the monopolization of seeds (Pollan, 2001). Indeed, inequitable distribution of political power is at the root of public distrust of genetically engineered foods (Nestle, 2004). That's why patents on seed varieties, identifying GMOs on food labels, and international trade agreements are frequently mentioned in the debate as well.

GENETIC ENGINEERING OF MICROORGANISMS

Microbial GE involves the exploitation, genetic manipulation, and alterations of microorganisms to make commercial valuable products and that also involves fermentation and various upstream and downstream processes (Table 12.4).

Microorganisms produce an amazing array of valuable products such as macromolecules e.g., proteins, nucleic acids, carbohydrate polymers, or smaller molecules. Such products are usually divided into metabolites that are essential for vegetative growth (primary metabolites) and those which give advantages over adverse environment (secondary metabolites). They usually produce these compounds in small amounts that are needed for their own benefit.

The genes encoding individual enzymes of antibiotic biosynthesis which have already been cloned include those of the cephalosporin, clavulanic acid, prodigiosin, undecylprodigiosin, actinomycin, and candididin pathways. The isopenicillin N synthetase ("CyClaSe") gene of *Cephalosporium acremonium* has been cloned in *Escherichia coli* and expressed at a level of 20% of total cell protein. Cyclase gene of *Penicillium chrysogenum* and *Streptomyces clavuligerus* has also been cloned in *E. coli* system. The expandase/hydroxylase gene of *C. acremonium* has been cloned in *E. coli*. The protein accumulated as inclusion bodies in *E. coli* near to 15% of total cell protein.

Similar to *E. coli*, nowadays *Bacillus subtilis*, *Pichia pastoris*, *Saccharomyces cerevisiae* have also emerged as a promising heterologous expression system for prokaryotic and eukaryotic candidate genes.

The potential applications of microbial GE is the production of pharmaceuticals, nutraceuticals by bacteria or other microorganisms that produce

TABLE 12.4 Important Genes Could Be Transferred by Selected Methods into Microorganisms for the Improvement of Product and Industrial Application

Type of Organism	Industrial Applications	Gene	Gene Transfer Methods
<i>Aspergillus</i>	Food fermentations	<i>asparaginase gene; afp</i>	Protoplast transformation
			Electroporation
			Biolistic transformation
<i>Bacillus</i>	Industrial enzymes	Late competence gene (<i>Com</i>)	Transformation of competent cells
	Fine chemicals	<i>Amy α</i>	Protoplast transformation
	Antibiotics	<i>bacD; fenD; sfp; ituD; bacA</i>	Electroporation
	Insecticides	<i>cry</i>	Electroporation
<i>Corynebacterium</i>	Amino acids	<i>betP; PurD</i>	Protoplast transformation
			Conjugation
			Electroporation
<i>Escherichia coli</i>	Therapeutic protein production	<i>ins, il2, hGH, ifnβ, etc.</i>	Transformation of competent cells
	Biodegradable plastics	<i>phbA, phbB, and phbC</i>	Transformation of competent cells
Lactic acid bacteria	Food fermentations	<i>agl, glgP, α-amy, malP, dexC, mall</i>	Electroporation
	Organic acids	<i>ldh; adhE–Ldb1707; ccpA-pepR1</i>	Protoplast transformation
<i>Pseudomonas</i>	Plant biological control agents	<i>pca; dapG; prn; plt</i>	Electroporation
	Bioremediation	<i>xyl; tod; etc.</i>	Conjugation
<i>Streptomyces</i>	Antibiotics, antitumor	<i>Str; cagA; sgcA; and sgcB</i>	Protoplast transformation
	and antiparasitic agents	<i>aves 1–4</i>	Electroporation
	Herbicides	<i>bar</i>	Conjugation
	Insecticide	<i>choA</i>	Conjugation
Yeasts	Food and beverage fermentations	<i>leu2; sta1, dex1 (=sta2), and sta3, etc.</i>	Protoplast transformation
			Electroporation

economically, clinically important products like human insulin for diabetics or human growth hormone for dwarf individuals. Techniques are being perfected to transfer human genes into cows, sheep, and goats to obtain medically significant products from the milk of these animals.

Bacteria can be genetically altered to emit a green fluorescent protein visible in ultraviolet light when they metabolize the explosive TNT leaking from land mines. Researchers envision a day when bacteria can be applied to a tract of land with a crop duster and then be analyzed from a helicopter. Using various biotechnological processes, genes can be added from other organisms that will confer the ability to degrade toxinogenic chemicals such as toluene, commonly found in chemical and radiation waste sites.

GM microorganisms can be used a living sensor to detect any particular chemicals in soil, air, or other inorganic or biological specimens. The genetic manipulation in the bacterium *Deinococcus radiodurans* can enhance the performance of bacterial potential to clean-up toxic-waste sites.

Ethical Issues and Public Perception

Genetically engineered organisms, unlike toxic chemicals and other noxious pollutants, but like all other living creature, can increase their populations and spread far and wide both in space and time. This is the first characteristic of deleterious chimeric microorganism that causes consternation. It is not unreasonable to assume that a bacterial plasmid splice to a viral genome will be disseminated in nature at faster rate than the naturally occurring bacterial species. These hybrid DNA molecules may carry potentially harmful genes (such as resistance to a miracle drug) then tremendous dangers could be anticipated. The use of viral genomes that cause cell transformation and tumor formation was looked upon with aided trepidation. Would an SV40 DNA accidentally integrated in that of a human lead to malignancy in the latter nonpermissive host?

The EPA has authority to regulate GMOs under the Toxic Substances Control Act (TSCA). The TSCA authorizes the EPA to regulate chemical substances that may present an unreasonable risk of injury to health or the environment. Manufacturers of covered substances must submit a premanufacture notification to the EPA. The EPA has determined that GMO microorganisms are chemical substances subject to regulation under the TSCA. The EPA has established regulations specifically for microorganisms that require submission of a Microbial Commercial Activity Notice (MCAN) before they are used for commercial purposes. The Notice must include information describing the microorganism's characteristics and genetic construction; byproducts of its manufacture, use, and disposal; health and environmental effects data; and other information.

The National Environmental Policy Act (NEPA) requires federal agencies to prepare Environmental Assessments (EAs) of their actions which may include adopting a policy or approving a permit. The purpose of the EAs is

to determine if such actions are likely to significantly impact the environment. If a federal action is likely to have a significant impact, the agency must prepare a more detailed evaluation called an Environmental Impact Statement (EIS). Federal agency approvals of GMOs may require an EA or an EIS in some circumstances.

State law generally plays little role in the regulation of GMOs in the United States. The federal preemption doctrine, which bars conflicting state regulation when Congress intends federal regulation to occupy a particular field, precludes many aspects of state regulation of GMOs (Farquhar and Meyer, 2007).

A rare example in which one state's law is more stringent than federal law on GMOs involves a bioengineered tropical aquarium fish known the GloFish, which is unregulated at the federal level but has been banned by the California Fish and Game Commission (Kenneth, 2003).

Biological risks may also accompany the reason of engineered microorganism that is judges on the basis of available data to be safe, but actually turn out to be hazardous. It is argued that splicing of DNA from species that do not exchange genetic matter in nature may be a trigger off abnormal expression or repression of gene in the heterologous host. It is this unknown nature of biological risks which has taxed the minds of expert and the lay public alike.

A biological hazard that one cannot afford to overlook is that of using engineered organism for biological warfare. Undoubtedly, microbes are far more cost-effective as exterminators than even the deadliest of explosive. A biological weapon will not be restricted like nuclear weapons to nations that are sufficiently affluent and possess the scientific know how. Any nation can create an arsenal of microbial killers, with a minimum of expertise and resources. One of the main deterrents, however, for using genetically engineered microorganism for biological warfare in the current lack of methods required to confine the spread of the agents only to defined population (the enemies). Besides, it is still cheaper and easier to use naturally available deadly organisms such as the food poisoning bacterium (*C. botulinum*), the tetanus bacterium (*C. tetani*), or the encephalitis viruses, than undertake the expense and labor of creating chimeric monsters.

Further understanding of molecular biology and lack of any obvious catastrophe even after two decades of rDNA research has lessened the intense alarmist attitude in the majority of the scientific and lay communities. Contaminants of chimeric organisms as description in the types and goals of a research were deemed to be sufficient retardants for gene cloning to go out of hand, both physically and metaphorically.

PERCEPTION OVERVIEW THEORETICAL

An individual's perception of the risks and benefit of a new technology is determined by personally selected source of information, values, interests,

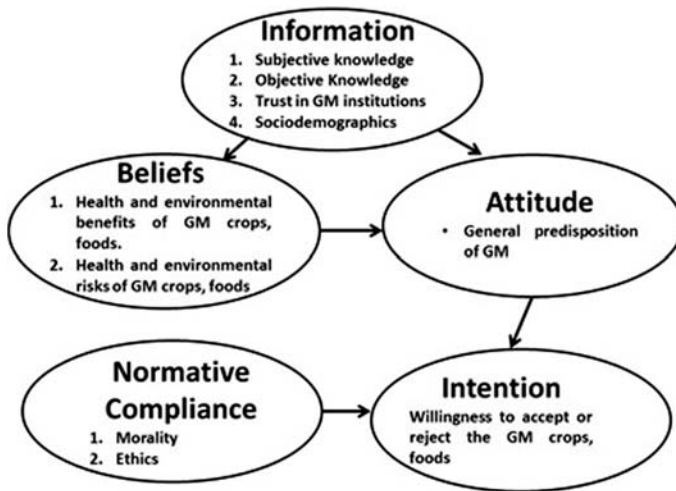


FIGURE 12.3 Basis of theoretical framework for the analysis. The information and belief generated on the basis of background, religion, general knowledge, and scientific knowledge. The information indicated the attitude and behavior of people toward goal and risk concerned with GM crops and foods. The overall intention generates acceptance and rejection of genetic engineering.

and individual experience (Hoban, 2004). In the case of genetically engineered organisms and food, most people cannot count on their own experience. Instead, they have to rely on information distributed through the mass media by representatives from industry, government, public interest groups and academics. Based on socially communicated values, social status, and professional affiliation, a person regards particular sources of information to be more reliable than, others. The selection of the sources of information is also strongly influenced by his/her personal worldview and interests. According to Fishbein's multiattribute model, a person's attitude toward any object is a function of his/her beliefs about the object and the implicit evaluative responses (or aspects) associated with those beliefs. In this regard, a stakeholder's perception is his/her attitude in favor of or against GM crops/food as used in this research. The theoretical framework for the analysis is presented below (Fig. 12.3).

After discussing present aspects regarding public perception and ethical issues we propose that rational studies must be required by Government, Institutions and concerned agencies. The use of multiple item scales is a vast improvement over the more usual reliance on single questions. By including many measures of the same concept, it becomes possible to:

1. **Test** the assumption that they measure what you assume they do using factor analysis and other sophisticated statistical procedures;
2. **Refine** the measurement by excluding items that show statistical or conceptual weaknesses;

3. **Reduce arbitrariness** by relying on the average of several items rather than putting all your eggs in one basket by choosing a single question which may be unrepresentative in ways you could not anticipate; and
4. **Reduce random measurement error** that inevitably arises from the statistical imprecision of any single item.

The cumulative impact of these advantages is so great that the use of single item measures of attitudes, values, or perceptions can rarely be justified in serious work.

Reframing Ethical Issues for Public Policy

There are inherent conflicts involved in how biotechnology develops as an industry and the way ethical questions and public policy positions are discussed and adopted. Key factors include, for example:

- How to define and evaluate man's fundamental *long-term* relationships to his genetic heritage, other species and his ecological system;
- How to address the pressure to *rapidly* advance scientific research and to exploit new product applications as they emerge;
- What priority to grant commercial needs to *quickly* realize a high return on a very expensive investment in a turbulent marketplace.

The conflict between the ethical issues that emerge as research proceeds and discoveries are made, and the time and other pressures to immediately move products to the marketplace create public policy issues that cannot be easily resolved for a number of complex and interacting reasons:

Potential health, economic, and business benefits are huge. The potential human and financial rewards that could emerge from curing serious diseases, increasing the food supply, and substantially extending and improving the quality of human life are very large. It is this possibility that drives researchers, investors, and potential benefactors.

Biotechnology/bioethical issues are not simple. The underlying science is complex, as are the resulting issues. Bioethics is a new field that is developing right along with biotechnology.

It is difficult to know which biotechnology-induced changes in an organism or production technology might result in large scale social or economic changes. The often new relationship of the discovery to the greater environment, human health, marketplace, and to future generations is unknown. The law of unintended consequences is a major concern.

Measurements of the socio-economic and market effects of a new technology are hard to make. Methods for measuring expected human, ecological, industrial, and financial risks, short and long-term costs/benefits, and other relevant factors are just being developed. It may be particularly difficult to

estimate the long terms costs of biotechnology innovations, given their often unpredictable effects.

There is pressure to achieve immediate short term economic gains that might have essentially unknowable long-term effects. For example, patenting corn, rice, potatoes, and wheat and the accompanying farming and marketing methods might reorder the entire agricultural industry and rural life.

Issues are set within conflicting time horizons and value systems. Research and marketing time horizons are relatively short, emphasizing immediate financial pay-off and scientific prestige. In contrast, bioethics and public policy questions often involve a long-term time horizon (generations of people), whole systems (ecological or industry), and the quality of individual and community life.

The definition of what “safe” means and how to evaluate an acceptable level of risk is still evolving. For example, how should manufacturers label bioengineered products and other products that may use genes inserted from plants to which people might be allergic? The large scale availability of genetic testing and its implications for the workplace and for inherited health problems are issues that are just now being addressed.

There is strong competitive pressure to go forward with new and potentially risky technology in a global market. European, Asian and other nations are fiercely competing with each other to develop and dominate a segment of the biotechnology industry, if not the industry itself.

CONCLUSIONS AND FUTURE ASPECTS

Most advances in Science and Technology with the potential of disturbing the health or survival of humans and our environment have raised controversies regarding the extent to which we should tolerate further research that could harm us in some way. In no case have these fears been more turbulently expressed than in those which are awakened by recombinant DNA technology. GE involves redesigning the basic blue prints of life in a deliberate manner. This has given rise to grave concerns about indiscriminate release of recombinant DNA containing organisms into the environment. Understandably, the immediate worry revolves around biohazards, due the presence of chimeric or synthetically reorganized organism in the environment at large. It has become quite apparent now that much more is at stake than immediate health and ecological imbalance. As already discussed in the preceding sections, this puts at risk our gene pools and ecosystems, as well as the global economy, social values and legal structure of our existing systems. Are these risks worth it? It is certainly too early to say this. Also we cannot really have a blanket ban on all uses of recombinant DNA technology, nor can we simply approve everything in the name of science. A technology that can potentially redesign even human beings will surely raise

moral, ethical and social dilemmas of a magnitude that the human race has never encountered before.

Public perceptions of GE/GMOs have generally been better evaluated in industrialized countries compared to developing countries. Public attitudes on adult and ESC research are positive, as a great part of the public adopts utilitarian ethical positions. Human reproductive cloning is generally seen very negatively. Disease associations support ES research. Debates on the production of embryos through nuclear transfer techniques (“therapeutic cloning”) are very intense in countries encountering a high influence of religious groups, such as the Roman Catholic Church and American White Evangelical Protestants. Church members often adopt less rigid views than their organizations. In Japan, India, and China, ESC research and “therapeutic cloning” are not of religious concern. As yet, the general public adheres to quite positive views on biotechnological innovations—but for GM crops and food the picture is quite different. Some farmers have adopted the technology while other farmers, mindful of the controversy surrounding the GM products and the threat of no markets for their crops have hesitated to use GM crops as parts of their agricultural operations. The public at large is also divided in its perception of such foods.

The debates and controversies have yet to resolve themselves into unanimous or even consensus opinion regarding the future of GE research. Discussions between experts, who apparently know that they are talking about, and the lay public, which may or may not understand fully the implications of the new technology, need to be fostered. The many crucial decisions which will certainly be made in the near future will affect the long-term future of our race as well as our genetic resources. Such decisions should be based on the best scientific information and active cooperation of the general public in order to allow effective choices for policy options.

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Chapter 13

The Way Ahead

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INTRODUCTION

The word “Agriculture” is derived from Latin word “agricultura” where ager means field and cultura means cultivation or growing. Agriculture is the cultivation of plants, animals, and fungi for food, fiber, biofuel, medicinal plants, and other products used to sustain and enhance human life. Agriculture was the key development in the rise of sedentary human civilization, whereby farming of domesticated species created food supplies that nurtured the development of civilization. Over one-third of the world’s workers are employed in agriculture, second only to the service sector. Agricultural production is required to feed the present population and keep open the option potential to feed the future growing population. It is a global challenge to produce required food for the growing population at affordable price. To meet the requirement of food for growing world population, large-scale production of crops and livestock products using chemicals, fertilizers,

pesticides, fossil fuels, high-yielding varieties (HYV), and various mechanization turned into industrialized agriculture. In this process, there has been degradation of natural resources like land, water, and air adversely affecting the environment and human health. There has also been increase in cost of production and deterioration of quality of food. For this reason, there has been a trend globally to move in the direction of sustainable agriculture. Sustainable agriculture is the production of food, fiber, or other plant or animal products using farming techniques that protect the environment, public health, human communities, and animal welfare. The practice raises crop and livestock causing least inflict on environment and maintains ecological balance. Agricultural production is needed to meet the food requirement in terms of quantity and quality of present growing population at affordable price and keep the natural resources sustainable to meet the demand of food for future population. Application of biotechnological tools has the potential to achieve the goal of sustainable agriculture. The Convention on Biological Diversity defined biotechnology as “any technology application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.”

CHALLENGES IN AGRICULTURE

The challenges in agriculture are scarcity of natural resources like land and water, population growth, poverty and hunger, and climate change. As per estimates of United Nation Population Reference Bureau, the current world population is 7.5 billion and will reach to 9.9 billion in 2050. To meet this demand, the world's food production is to be doubled. Food and Agriculture Organization (FAO) estimated that more than 800 million people in the world do not have enough to eat, causing 24,000 people to die every day from hunger, three quarters of whom are children under five. Not only quantity of food is a concern but also the quality of food matters. “Hidden hunger” or micronutrient deficiency of iodine, iron, or vitamin A is a serious concern. According to the Micronutrient Report, nearly 20% of the population (in the developing world) suffers from iodine deficiency, about 25% of children have subclinical vitamin A deficiency, and more than 40% of women are anemic. Scarcity of natural resources like land and water poses a great challenge to sustainable agriculture. Shrinkage as well as degradation of agricultural land, conversion of agricultural land to urban uses is a serious problem and horizontal extension of agricultural land is ceased. It is further aggravated by saturation of many crop yield production. Decrease in quantity and quality of water is also adversely affecting the agricultural production. The cost of agricultural production has been ever increasing and in developing country, it is exceeding beyond the purchasing power of the poor. Agriculture is the largest single cause of global warming. Three main contributory factors in producing greenhouse gases are fossil fuels, land use, and agriculture. Methane is released from rice cultivation and rumen

fermentation of animals, deforestation is linked to carbon dioxide release, and fertilizer application releases nitrous oxide. These agricultural processes comprise 54% of methane emission; roughly 80% of nitrous oxide emissions and virtually, all carbon dioxide emissions are related to land use. Intergovernmental Panel on Climate Change predicts a rise in global temperature of 1.1°C to 6.4°C by the end of the century. Estimates show that each one degree rise in temperature will cause grain yield to decline by 5%, posing a serious threat to food security. On the one hand, by adopting agricultural operations which are climate resilient and increasing forest cover which in turn reduce carbon emissions, on the other hand, we can step up food production to feed the world's growing population (FAO, 1994).

APPROACHES AND STRATEGIES OF SUSTAINABLE AGRICULTURE

The Golden Triangle of sustainable agriculture is environmental protection, economic growth, and social justice. The approaches are directed to maintain soil quality, reduce soil degradation and erosion; to save water; to reduce use of chemical fertilizers, pesticides causing less pollution; to produce large quantities of healthy food at low cost; to maintain biodiversity and address food security ensuring access to quantity and quality food to the consumers. The approaches should be holistic in nature involving management of agricultural inputs, genetic manipulations, innovative farming practices, social and policy decisions.

SOIL MANAGEMENT

We all know that “Healthy soil produces healthy crops.” Protecting and nurturing soil health is the prime importance in sustainable agriculture. Soil erosion is a major threat in soil health. Measures to reduce soil erosion include reduction or elimination of tillage (“zero tillage”), irrigation to reduce run off, and avoidance of traffic on wet soil, keeping soil covered with plant or mulch. Applications of compost and/or organic manures maintain the moisture in soil and thus reduce soil erosion. Cover crops stabilize agroecosystem by holding soil and nutrients in place, conserving soil moisture with mowed or standing dead molds, and increasing the water filtration rates and soil water holding capacity. Soil health is maintained with reduced use of chemical fertilizers and pesticides in sustainable agriculture (UC-SAREP, 2015)

WATER CONSERVATION

Use of water in agriculture operations accounts for nearly 70% of the water used throughout the world and the majority portion is used for irrigation. Water is a finite resource, the availability of which is declining with each

passing day. Use of large quantity of water causes ground water depletion and contamination from fertilizers, chemicals, and pesticide. Water scarcity is a major problem in dry areas and unpredictable and less rainfall due to climate change posing other problem to agriculture ([UC-SAREP, 2015](#)). The way ahead for water conservation includes use of improvised water management system. Water management is practiced with application of small quantities of water irrigation at crucial growth stages of the crop. The following efficient water management technologies may be adapted to attain sustainable agriculture. Proper land leveling increases the water application efficiency which leads to higher yields as well as increase in water use efficiency. It also has a direct bearing on the nutrient use efficiency. Adopting innovative methods of irrigation may save a considerable amount of water. Surface flooding irrigation method is an inefficient manner of using water. About 10% to 15% efficiency in water use can easily be achieved by adopting the appropriate methods of irrigation. The selection of the right method of irrigation is influenced by the soil type, land topography, crops to be grown, quality and quantity of water available for irrigation, and other site-specific variations.

Methods of Irrigation

Surface irrigation (flood irrigation): It is most common form of irrigation where water is applied and distributed over the soil surface by gravity. Three major types of surface irrigation are level basin, furrow, and border strip. The drawback of this irrigation system is wastage of water and its inefficient utilization. In level basin, water is applied rapidly to the entire basin and is allowed to infiltrate. Furrow irrigation method is generally used to irrigate row crops and vegetables. Furrow irrigation or its modified version viz. raised bed system can save irrigation water. The practice of alternate or skip furrow irrigation can save considerable quantity of water without compromising the agricultural yields in areas where water for irrigation purposes is scarce. In areas requiring surface drainage or where the cultivated crops are sensitive to water logging, the furrows are effective in removal of excess water. Excessive water intake and deep percolation losses are the major disadvantages of irrigation through furrows and border strips methods.

Surge flow irrigation: In surge flow irrigation, water is applied intermittently in a series of on and off modes of constant or variable time spans. It has the potential of reducing intake and percolation losses, increasing the irrigation efficiencies, and conserving irrigation water.

The pressurized method of irrigation like sprinklers and drip (Trickle) gives many advantages over the gravity surface irrigation methods in terms of water savings and yields. The quality of production through drip irrigation is generally superior to conventionally irrigated crops. These pressurized irrigation systems are very useful particularly in undulating and uneven lands or very coarse textured soils.

Fertigation: It is also possible to supply the nutrients to the crops through the pressurized system of irrigation popularly called as fertigation. Supplying liquid soluble fertilizers through a drip system can lead to savings in fertilizer applied to the extent of at least 40% without affecting the yield and a much higher application efficiency compared to the conventional methods. This is in addition to the savings in water applied which could be anywhere from 50% to 70%. It also produces superior quality produce. The stupendous progress in information technology coupled with the rapid advancements made in Geographical Information Systems, simulation tools, sensors, precision farming, and remote sensing has opened up new arena for water resources development and management. These tools should be an integral system of scientific management of irrigation networks, water distribution, crop planning, and related operational activities as they will enable the system managers to take correct and timely decisions. These tools can be equally, if not more effectively, utilized in assessment and monitoring of watershed-related development studies. Marker Assisted Selection, gene pyramiding, etc. can enable identification as well as introduction of genes (single/multiple) that can enhance water use efficiency as well as increase tolerance to water logging, soil salinity, or heavy metal toxicity. Use of drought-tolerant crop species, managing crops to reduce water loss, and “no planting” are also some of the mitigating approaches. Water salination and contamination of ground and surface water by pesticides, nitrates, selenium, etc. are also a serious threat. Use of salt tolerant crops and low volume irrigation is the corrective measure. Rain water harvesting and water shed management are other measures to conserve water. Rainwater harvesting is the process of collecting, concentrating, and improving the productive use of rainwater, and reducing unproductive depletion such as runoff, evaporation, and seepage. Harvested rainwater may be used for providing presowing irrigation, supplemental/life saving irrigation, recharging ground water, and/or for domestic purposes.

In Situ Water Harvesting Options

Land leveling and field bunding: Leveled plots with thick, strong, and raised bunds impound rainwater in situ; land leveling helps rainwater distribution in the entire field in an even and uniform way.

Land shaping (trenching, providing directional slope, etc.): To concentrate runoff at the desired location, such as tree basins in orchards land shaping for flowing water.

Microcatchment water harvesting: By converting flat plots into ridge-furrow system, crops are planted in furrows so that rainwater concentrates in planting area and root zone. It improves moisture conservation and enhances crop yields.

Raised-sunken bed technology: For interplot rainwater harvesting, high water requiring crops (e.g., rice) are cultivated in sunken beds and low water

requiring crops (e.g., maize, soybean, vegetables) on raised beds. It is very useful in high rainfall areas.

Terracing: On sloping landscapes and cultivating crops requiring well-drained conditions (maize, soybeans, vegetables, etc.), on upper terraces and high water demanding crops (rice, vegetables), on lower terraces where runoff concentrates. Divert water from upper terraces to lower terraces.

Ex Situ Water Harvesting Options

It needs a suitable storage structure and water conveyance system. A suitable reservoir is constructed to collect and store excess rainfall/runoff, stream flow, spring water, roof-top water, etc. Design of a water storage structure at microwatershed level depends on the following factors: amount of rainfall, characteristics of the catchment area that generates runoff, amount of runoff generated, command area to be irrigated with stored water, crop water requirement, etc. (Rattan & Biswas, 2014).

AIR QUALITY

Agricultural operations like agricultural burning, dust from tillage, burning of fossil fuels, pesticide drift from spraying, and nitrous oxide emissions from the use of nitrogen fertilizers adversely affect the air quality. It can be mitigated with sustainable approaches like incorporating the crop residue into the soil or making compost, using nonrenewable source of energy, using appropriate level of tillage, and planting wind breakers and cover crops (UC-SAREP, 2015).

ENERGY UTILIZATION

It is estimated that sustainable agriculture needs 30% less energy per unit of crop yield than that of industrialized agriculture. It reduces reliance on nonrenewable energy sources like fossil fuel, petroleum, and less pollution to environment. Sustainable agriculture encourages doing the agriculture operations with renewable sources of energy like solar, wind, biomass, tidal, geothermal, small-scale hydro, biofuels, and wave-generated power or labors to the extent that is economically viable. These renewable resources have a huge potential for the agriculture industry. Use of various renewable energy sources in agricultural operations should be propagated and farmers should be provided with the subsidized equipment. Solar-operated water pumps and electricity unit, dryers for postharvest processing, and water heaters and greenhouse technologies are available for agricultural operations. Submersible solar photovoltaic water pump is cost-effective and causes less pollution to environment in comparison to generators run on fossil fuel. Use of renewable energy technology such as greenhouses for maintaining the optimum temperature for the growth

of plants and vegetables in cold climatic zones should be adopted to make agriculture sustainable (Chel & Kaushik, 2011).

BIOTECHNOLOGY FOR SUSTAINABLE AGRICULTURE

Biotechnology has been contributing to sustainable agriculture in the following ways:

- increased resistance against biotic stresses such as against insect pests and diseases;
- increased resistance against abiotic stresses such as against drought, cold, flooding, and problem soils;
- bioremediation of polluted soils and biodetectors for monitoring pollution;
- increased productivity and quality of agriculture produces;
- enhanced nitrogen fixation and increased nutrient uptake and use efficiency;
- improvement in fermentation technology;
- through technological improvement for generating biomass-derived energy; and
- through generation of high nutrient levels in nutrient-deficient staple crops such as rice.

The contribution of biotechnology to achieve sustainable agriculture is envisaged by means of reducing the use of agrochemicals, chemical fertilizers, pesticides, etc. through genetic manipulation of the plants with resistance to biotic and abiotic stresses. Selected genes from different genetic resources are integrated in desirable genotypes. By means of systematic pyramiding of genes, integration of desirable genes in one genotype for different traits, viz. tolerance to stresses, productivity, and nutritional quality is achieved. Technology, including new varieties and breeds, is an essential element of sustainable agriculture. Apart from that, some nontechnological aspects also influence to achieve the goal of sustainable agriculture. These included governmental policy, institutional and infrastructural support, technology sharing and transfer mechanisms, and public awareness (Singh, 2000). Biotechnology is playing important role not only in achieving sustainable development of agriculture, animal husbandry, fisheries, and forestry but also in food and other primary product-related industries. It has tremendous potential to cast an impact on global food security, human and animal health, overall livelihood of mankind, and environmental health (Serageldin, 1999). Various interdependent components of modern biotechnology are genomics, bioinformatics, transformation, molecular breeding, diagnostics, and vaccine technology. Every component of biotechnology has its own potential and impact. Major controversies generally surround the transformation component resulting in genetically modified organisms (GMOs). Besides, there are other contradictions which are technology-transcending

and socioeconomic in nature (Leisinger, 2000). Biotechnology itself is not a controversial subject; however, its mode and nature of application, through techniques and technologies, could pose contradictions. Biotechnology, biodiversity, and sustainable agriculture are complementary, synergistic, and interdependent to each other. Judicious, rational, and science- and need-based exploitation of biotechnological manipulation of genetic resources in a judicious manner should be done to achieve sustainable agriculture. This is a double-edged weapon and when we deviate from the scientific ethic, the controversy arises (Singh, 2000).

The issues of food safety and biosafety are matters of real concerns. Biodiversity and sustainability face a serious threat from horizontal gene transfer through genetic engineering. However, the risks could be avoided or at least minimized with its scientific assessment and adoption of preventive and corrective measures (Singh, 2000). The molecular tools of biotechnology have accelerated precision breeding by identifying, isolating, cloning, and transferring desired genes from one species to another. All the processes of genetic manipulations like collection, conservation, utilization, and evaluation have been predominantly influenced by biotechnology. DNA libraries are a major supplement to germ plasm conservation, let alone various in vitro conserved materials. In vitro conservation of plant species that are asexually propagated or produce recalcitrant seeds or produce recalcitrant seeds or are infertile is an important biotechnological approach. Germ plasm conservation strategy in animal has been greatly strengthened by cryopreservation of semen, embryos, and even somatic cloning (Singh, 2000).

Genetic Engineering is the process of manipulating an organism's genetic material including genes from other species in an effort to produce desired traits such as pest resistance or drought tolerance. The term GMOs is used for those plants, animals, or microorganisms that underwent change through genetic engineering. Plants are modified for insect protection, herbicide resistance, and virus resistance and enhance nutrition, tolerance to environmental stresses, and the production of edible vaccines. In this process, crops are developed that mature faster and produce resistance or tolerance to biotic and abiotic stresses. Important crop traits targeted with biotechnological tool include resistant to plant fungal and viral diseases, drought, and heat tolerance. Researches are directed on use of genetically modified plants fortified with nutraceuticals, pharmaceuticals, and oral vaccine expressed in plants-fruits and vegetables for direct consumption, production of commercially valuable protein in plants such as spider silk protein and polymers that are used in surgery or tissue replacement. Genetic engineering on tobacco plant produced the first protein pharmaceutical-human growth hormone in 1986 and antibody in 1989. Genetically modified animals are used for research, animal models, and the production of agricultural or pharmaceutical products. The genetically modified animals include animals with genes knocked out, increased susceptibility to disease, hormones for extra growth, and the ability

to express proteins in their milk. Through genetic manipulation, scientists are able to make salmon to grow larger and faster and resistance to mad cow disease was enhanced in cattle. Transgenic animals are routinely bred to carry human genes or mutations in specific genes, thus facilitating the study of the progression and genetic determinants of different diseases (Phillips, 2008).

Conflicts and Way Out

There are concerns about risks posed by some aspects of biotechnology. In the context of biodiversity and sustainable agriculture, the technology-inherent concerns are reduction of biodiversity and poor access to tailored genetic resources, adverse effects on human health and environmental degradation. The other technology-related concern is emergence of increased economic inequity, inability to access to the new and emerging technologies and products. This would be more apparent for resource-poor people in developing countries. Genetic vulnerability may be increased due to replacement of diverse traditional resources with selected GMOs. Rather, biotechnology could be used for increasing biodiversity primarily through the channeling of genes from wild and weedy relatives into cultivated forms (Singh, 2000).

Horizontal gene transfer to unwanted sources leading to the development of more aggressive weeds or wild relatives with increased resistance to environmental stresses or diseases would cause both genetic erosion and ecological imbalance. The loss of fish diversity associated with the escape of cultured transgenic fish and its mating with its wild counterpart appears to be a real threat. But, efficacies of such studies need to be ascertained more realistically before reaching definite conclusions (Singh, 2000).

The current trend of biotechnology development has generally been pro-rich as most of the biotechnological research and its application is in the hands of private sectors of developed countries, thus widening the gap between the rich and the poor. This trend is a hindrance to achieve sustainable global development. This contradiction can be resolved if the pro-poor features of biotechnology are promoted. The public sector in developing countries must have the responsibility and capacity for the promotion of pro-poor features of modern biotechnology.

The contradictions and risks associated with the development and application of biotechnology should be addressed by the individual countries in a scientific way with necessary research, impact monitoring, technology refinement, technology assessment, and adjustment capacities as per their requirement.

Biosafety policies and measures would thus have serious implications for the use of biotechnology for sustainable agriculture, food security, and biodiversity. For this purpose, each country, developed and developing, must have adequate and effective biosafety rules, regulations and legislations, capacity for detailed risk assessment and management, and mechanisms and instruments for monitoring the use and compliance of biosafety measures.

Introduction or import of GMOs and other genetically engineered products either through private or through public sector channels should adequately be covered under legislation and handled with great care because of the threat of introducing completely new organism or genetic material.

Securing Benefits for Developing Countries

Developing countries need exploitation of the products and findings of biotechnology applications for their welfare. For example, production and distribution of in vitro-cultured disease-free plantlets are already benefiting small farmers in developing countries. The virus-resistant papaya transgenics developed in Hawaii are being shared with developing countries. Some of the international associations and agencies such as International Service for the Acquisition of Agro-Biotech Applications are already assisting in sharing biotechnology products and techniques between developing and developed countries. Regional and international initiatives such as those by the Consultative Group on International Agricultural Research, FAO-supported regional biotechnology and research institutions and associations such as Asia Pacific Association of Agricultural Research Institution and the Global Forum for Agricultural Research should be further strengthened to undertake collaborative activities. Several countries lack basic research and technology development resources and infrastructure to even absorb introduced technologies, let alone the generation of new knowledge and technologies (Singh, 1995). Such countries must give high priority to develop the minimum facilities. FAO and other UN agencies and donors should assist developing countries in building capacity for harnessing the latest developments in the field of biotechnology. Free access to information at all levels is fundamental to the rapid improvement of crop, livestock, forestry, and fish species. This is particularly important for the developing countries which are not in a position to generate new technologies but are in a position to use them. Many of the ethics-related issues, such as cloning of mammalian species, are being debated in the context of Intellectual Property Right (IPR) legislation and religious and cultural settings (Singh, 2000).

The global food production needs to keep pace with the increase in world population and currently, it needs to double the future requirement. The negative impact of the Green Revolution may be summarized as bypassing the vast rain fed and dry land areas and commodities, thus exacerbating inequity. Environmental degradation and depletion of soil and water resources and quality caused due to inefficient and excessive use of irrigation, fertilizers, and other agrochemicals and buildup of pesticide resistance in major pests, and loss of land races and overall erosion of biodiversity leading to greater genetic vulnerability. In the present scenario, the option for horizontal expansion of cropped area in most developing countries is almost closed; so, the way ahead should be directed toward sustainable intensification of

agriculture. The Green Revolution path of agricultural intensification during the past over 30 years was certainly the most effective path to overcome the problems of widespread food insecurity and hunger. However, Green Revolution has many shortcomings. In Green Revolution, varieties were developed using conventional Mendelian approaches whose impact is plateauing off, yield ceilings have been attained in the HYVs, and the approach had limited success in designing crops tolerant to complex stresses, such as drought (Singh, 2000).

The way ahead must therefore seek the development of highly productive, efficient, resistant (to biotic and abiotic stresses), remunerative, quality-rich genotypes suitable both for congenial (irrigated) and noncongenial (rain fed/dry land) settings, which when blended with time-tested traditional technologies and appropriate policies, and synergized with modern information technology, should promote congruence of enhanced productivity, sustained and healthy ecology and environment, referred to as ecotechnologies (Swaminathan, 2000).

Instead, the way ahead is to develop and promote propoor technologies which may enhance the income of poor people, improve their purchasing capacity and food self-reliance, and augment the production of their commodities and agroecological settings, altogether to improve their food security (Persley, 2000). Food security should mean not only calories and protein adequacy and balance but also adequacy of vitamins and especially vitamin A, zinc, iron, and iodine as well as balance of micronutrients to counter deficiency disorders prevalent in poor people. An estimated 180 million children, mostly in developing countries, suffer from the vitamin A deficiency that leads to two million deaths annually. Future food security strategies must address the issues of nutritional adequacy along with the issues of food security. In this context, the development of the “Golden Rice” holds great promise for Asian people where rice is the staple food alongside widespread vitamin A and iron deficiency, especially among children and women. Golden Rice is genetically transformed rice in which the transgenes enable the rice plant to modify certain metabolic pathways in its cells to produce the precursors of vitamin A, which otherwise was not possible. It is fortuitous that as we have entered the new millennium and were seeking a technological breakthrough which may spearhead agricultural production in the next 30 years at a pace faster than that during the past 30 years (the Green Revolution era), modern biotechnology with multiple and far reaching potential has appeared on the horizon. As mentioned earlier, it is already being used for and has the potential to enhance yield levels, increase input use efficiency, reduce risk and depress effects of biotic and abiotic stresses, and enhance nutritional quality leading to increased food security, nutritional adequacy, poverty alleviation, environmental protection, and sustainable agriculture (Singh, 2000).

Biotechnology should be kept in a balanced perspective by integrating it within the national research and technology development framework and

using it as an adjunct and not as a substitute for conventional technologies in solving problems identified through national priority setting mechanisms. The technology inherent and technology transcending risks or contradictions should be critically and scientifically assessed in a transparent manner. Capacities and measures should be in place to manage the risks, minimize the negative effects, and promote the positive impacts. Each country must have the necessary infrastructure, human resource, financial support, and policy for meeting the challenges and capturing the novel opportunities (Singh, 2000).

SUSTAINABLE FARMING PRACTICE

Many innovative farming practices are in operations to make the agriculture sustainable. Plants are grown that can create their own nutrients so as to reduce the need of fertilizer. Cultivation of plant with *Rhizobium* and *Azotobacter* where nitrogen fixation (biofertilizer) can take place is an important sustainable approach. Preparation and application of vermicompost is a major player in organic farming. The produces/products obtained from organic farming and animal husbandry practices are free from chemicals and considered healthy food and possess huge demand. Crop rotation practice suppresses weeds, pathogens, and insect pests; thus, it minimizes the use of pesticide. Cultivation of mixing crops reduces the risk of disease destroying a whole crop and decreases the need of pesticides and herbicides. Integrated crop and livestock operations are highly complementary both biologically and economically. According to an estimate, about half of the world's food comes from farms that raised both crop and livestock. Mixed farming like paddy-cum-prawn cultivation, duck-cum-fish farming, crop-sericulture, and agroforestry are symbiotic in nature and boost the income of farmers.

Diversified farms are economically and ecologically resilient. Loss of one crop or market fluctuations offers as an economic buffer to the producers. Diversification of crops including livestock and other cultural practices makes agriculture to be sustainable. Feed costs are the largest input in livestock operations. Feed from agricultural operations and by-products help reduce livestock feed cost. Mixed farming reduces the feed cost and utilization of agricultural by products. Feeding of nonconventional feed, urea molasses treatment of cereal straw to livestock and poultry reduces the production cost of animal products. Scarcity of grazing land and low quality of crop residues which are the major sources of available feed for ruminants are predominantly two major hurdles faced by livestock farming. Farmers should be encouraged to improved utilization of rice straw and other crop residues as a strategic solution to increase both the numbers and productivity of their ruminants, meeting the traditional demand for crop production and the increasing demand for livestock products. To be sustainable, an acceptable feeding system for improved rice straw utilization should be simple, machinery-independent, use cheap and freely available inputs, and easily

fit into the farmer normal routine. In this perspective, further research should be directed to treatment of straw with locally available inputs such as urea, urine, calcium oxide or calcium hydroxide, and their combinations. It is also important that protein-rich by-products such as fish meal, cotton seed, or oil cakes as well as necessary minerals and vitamins should be supplemented with treated straw to make it cost-effective and improve the feed quality. In addition, plant breeders should develop crop cultivars which can produce large amounts of high quality residues as ruminant feed without the expense of grain yield and quality (Trach, 1998).

Urea molasses multivitamin block was acknowledged as a safe and simple route for supplementing low-quality diets with nitrogen and minerals. There was also agreement that supplementation was essential to sustain yields and reproductive performance in dairy cows and buffalo (Wadha & Bakshi, 2011). Use of area-specific mineral mixtures is reported to enhance milk yield in dairy cattle in India.

In India and South-East Asia, there is an increasing interest in using Azolla feeding, a water fern, *Azolla caroliniana*, cultivated on water bodies as a good source of protein for cattle, goat, poultry, and fish. As a part of strategic feeding practice, the fern is mixed with protein deficient elephant grass, *Pennisetum purpureum* to make it a balanced feed. The Azolla has been traditionally used as a fertilizer for rice paddies. A positive note was the introduction of spineless Cactus as a potential forage source in arid and semiarid areas. Production and utilization of forage from trees, particularly in Africa and Southern Asia, is also an alternate feeding option. Research had shown how to reduce the adverse effects of tannin in some leaves (e.g., Calliandra) by adding polyethylene glycol at feeding. Mixed farming provides an opportunity to recycle the residues thus offer an efficient disposal as well as economic benefits. Poultry manure is commonly used for ruminant feeding and poultry and pig manure can be used to generate algae as a feed for fish. Slaughter house wastes, when adequately processed, make good source of protein (offal and viscera) and mineral (bones) supplements in animal feed. Kitchen wastes are commonly fed to pigs and industrial fish wastes are dried to produce fish meal for poultry.

SUSTAINABLE LIVESTOCK OPERATIONS

Livestock products such as meat, milk, and eggs provide a source of high-quality proteins and an important player to achieve food security. It is estimated that globally, one in seven human is undernourished, of which protein deficiency is a major contributing factor. Over the past decade, cereal yields per hectare have fallen in one-quarter of countries. Meanwhile, developing nation and the growing population are in more demand for animal protein. Livestock sector also provides wool, hides and skin, manure, draught power and transport. The sector serves as a source of income for many small and

marginal farmers in developing countries to purchase food as well as agricultural inputs like seed, fertilizers, and pesticides. It provides additional economic stability to the farm or household, serving as cash buffer in case of small livestock and as capital reserve in large animals. It also serves as deterrent against inflation. In mixed farming systems, livestock combats the risks associated with crop production. They are also considered as liquid assets that can be encashed during any crisis, adding further stability to the production system. Sustainable livestock sector has a great potential for employment generation. Dairying is a labor-intensive sector and its different activities like production, processing, and marketing provide job opportunities. Sheep, goat, poultry, and rabbit husbandry, especially in backyard production system, provides an important source of part-time job opportunities particularly for landless women and children. Livestock processing sector is also contributing in employment generation for the rural masses thus reducing their migration to cities. Livestock is providing renewable source of energy in the form of draught power for pulling agricultural implements, transportation, pumping irrigation water, and skidding in forest. Draught animals remain the most cost-effective power source for small- and medium-scale farmers. Use of animal draught power in agricultural operations is being considered as a sustainable approach. Dung is used as fuel source for households in Asia, Africa, and Latin America. It is also used as biogas production indigenously with minimum involvement of cost. In this way, dung serves as a substitute for fossil fuel or fuel wood for farmers in tropical countries and in turns; it reduces pollution and helps conservation of forest cover. Livestock operation acts as a source of organic fertilizer and soil conditioner. Nutrient recycling is considered to be an essential component of any sustainable farming system. The integration of livestock and crop farming allows for efficient nutrient recycling. Livestock uses the crop residue, such as cereal straws, as well as maize and sorghum stoves and ground nut haulms as feed. The manure produced by the livestock can be recycled directly as fertilizers for crop farming (Sansoucy, 2006). Recycling of crop harvest residue and stalk eliminates the incidence of their burning in field for easy disposal which in turn causing heavy environmental pollution in northern India in the form of smog causing respiratory distress to human and traffic disruption due to poor visibility.

As per an estimate, close to 1 billion of the world's poorest people rely on livestock for their livelihood. Sustainable animal husbandry practice includes selection of good genetic stock, quality germ plasm, health care and vaccination, processing, preservation, and value-added marketing of animal products.

Livestock production occupies 70% of all land used for agriculture, or 30% of the land surface of the globe. This sector is a major source of greenhouse gases, responsible for 18% of the world's greenhouse gas emissions as measured in CO₂ equivalents. The UNEP states that methane emissions from

global livestock are projected to increase by 60% by 2030 under current practices and consumption pattern. Research is directed to contain the methane production in rumen by manipulation of rumen microbial flora as well as adding of antimethanogenic feed ingredients. Some plant extracts can alter rumen microbial population to use nitrogen and energy more efficiently. This micromanipulation of rumen environment not only leads to more efficient meat and milk production but also leads to proportionately less emission of by-product greenhouse gas and ammonia. Research indicated that an enzyme in red clover, *Trifolium pretense*, widely grown in temperate countries, increases the ability of ruminants to utilize dietary protein more efficiently. It is shown in field trial that dairy cows fed on more clover in their diets increased their feed consumption and resulted in to more milk production. In Australia, during dry autumn, most pasture plants possess poor feed value sheep nibble on the deep-rooted perennial tar bush, *Eremophila glebra*, and get inherent natural benefits. Tar bush is reported to combat gastrointestinal nematodes and acidosis and reduces emissions of methane from rumen. High production should be achieved by using minimum number of high yielding elite germ plasm. The number of unproductive or poor yielding should be minimized in order to reduce methane production and utilization feed and fodders. It is argued that consumption of grain by the livestock acts as competitive inhibition to food security to human. About 70% of the grains used by developed countries are fed to the animals. Livestock consumes an estimated one-third or more of the world's cereal grains, with 40% of such feed going to ruminants mainly cattle. Sustainable approaches are aimed to use alternate feeds for the animals that do not compete with human food. Feeding of silage, hay, high-fiber crop residue that is unsuitable for human consumption should be considered. With improved breeding and cultivation, ruminant animals can yield food that is better for people and the planet (Eisler, Lee, & Tarlton, 2014).

SUSTAINABLE FOREST MANAGEMENT

Depletion and degradation of world's forest resources are a major concern and sustainable approaches are directed to mitigate them. Wood is the most versatile and useful economic product derived from forest. About half the wood consumed, plus some manufacturing residues, provides about 5% of world's energy consumption. In developing countries, about 80% of wood production is used for energy. Besides wood, forest offers other products such as foods, medicines, gums, latexes, fibers, dyes, and livestock fodder. Nonwood forest products facilitate opportunities for entrepreneurship development. The wildlife in forests contributes to food supplies and supports a substantial tourism industry. Forest and trees contribute in global food security in many ways: by direct supply of food products for man and animals, by increasing or diversifying family income so boosting purchase power

available for food, and by conservation of the resource base for agriculture. Forest is an important component of our ecosystem and provides shelter to more than 200 million people in the tropics. Forests conserve mountain watersheds, soil, and water, protect land from wind and water erosion, help prevent desertification, and modulate climate and sequester carbon, thereby buffering the global warming attributed to increasing levels of atmospheric carbon dioxide. Mangroves, in coastal areas, protect the land against soil erosion by the water as well as providing safe breeding atmosphere for fish and shrimps. The forests face severe threat in the form of deforestation and degradation in tropical and developing countries and quality decline in temperate and boreal forests. Tropical deforestation takes place due to agricultural expansion in all its forms, from shifting cultivation to cattle ranching; the need for this stems, in turn, from high population densities and growth rates. If we go through the deforestation trend, it is apparent that maximum deforestation took place in developed countries like the United States, Europe during 18th and 19th century when they had high population growth, agricultural areas were expanding, and industrialization was taking place. In the later stage, the developed countries were in position to reverse the deforestation trend when their dependence on fuel wood reduced due to use of coal and other fossil fuels as a substitute, stabilization of population growth and adoption of agricultural intensification and economic growth. Forest depletion in developing world is connected to poverty, lack of development, and excessive population growth. It is therefore logical to expect in reversal of deforestation trend when the developmental criteria are achieved as in case of developed nations. In recent times, forest cleaning is going on at unabated pace in most of the tropical countries as a consequence to economic development. The problem of deforestation and degradation can be managed by keeping it in line with land use plans and by maintaining sufficient trees and woodland within farming system. Residual forests must be brought under sustainable management approach to produce large enough income to provide actual alternatives to agriculture. Conflicting approaches to preserve the global forest cover are propagated. Environmental activists of developed countries are advocating sustained campaign against import of tropical timber which they believe that it will combat destruction of tropical forest cover. On the other hand, developing countries put forward the opinion that production and conservation can be persuaded simultaneously. Alternatively, specific forests can be reserved only for production and only for conservation (FAO, 1994).

SUSTAINABLE FISHERIES

The main reason for a decline in the productivity of many fisheries is due to the increased pressure on marine capture fisheries, from growing populations, rising demand for seafood, and a rapid increase in fisheries

exploitation. According to the UN FAO, 32% of fish stocks are now over-exploited, depleted, or recovering from depletion, and the situation is deteriorating every year. As a result, the sector is under stress to deliver less food and income and supporting fewer livelihoods than they had the potential. Moreover, the overexploited fisheries are also more vulnerable to external pressures such as climate change and pollution. However, research and consultation undertaken by the International Sustainability Unit Marine Programme show that practical solutions for these challenges are available. Wild fish stocks are of enormous importance to economic output, livelihoods, and food security. Contribution to global GDP can be increased in a considerable manner by rebuilding degraded fisheries and managing fishery activities in sustainable way. The transition of global fisheries to sustainable management not only secures these benefits for the long term but also increases productivity. Fisheries contribute approximately US\$ 274 billion to global GDP. However, they are currently an underperforming asset. The World Bank estimated that if fisheries were managed optimally, they could deliver an additional \$50 billion each year. Fisheries provide employment for hundreds of millions of people in direct and indirect ways. Millions of people in developing countries are involved in fishing activities and thus, the sector often plays a key role in preventing and reducing poverty. Only sustainably managed fish stocks can ensure the viability of these livelihoods and, following recovery, generate more employment in the long term. It goes beyond numbers as sustainable fisheries often also provide a better quality of employment with higher safety standards. Fish is a renewable and healthy food source especially protein to 1 billion people. Rebuilding fisheries provides direct economic, social, and food security benefits and the transition to its sustainable management would make marine ecosystems more resilient to the effect of climate change and pollution. Fisheries are vital components of ecosystems, and healthy ecosystems are pivotal to the continued productivity of fisheries. The biggest threat to marine capture fisheries comes from climate change and developing resource-poor countries is the most vulnerable to its adverse effect. Fishery sector has the potential to play a crucial role in achieving food security, offer sustainable livelihoods, and provide resilient economies by managing the fishery sector in a responsible manner (ISU Marine Programme, 2012).

Aquaculture faces challenges such as disease outbreak, feed production, and waste removal. A closed-contained system of aquaculture, either solid-wall systems that float on the water or tank systems that operate entirely on land, is successfully growing fish. By means of separating farmed species from native populations, it is expected to protect the environment from accidental fish escapes in these two systems, contain the spread and transfer of disease and parasites, and decrease the amount of fish feed and waste released into the local ecosystem (Jean-Michael Cousteau, 2014).

Strict monitoring and enforcement of regulatory mechanism are to be enforced to achieve the goal of sustainable fishery. It is estimated that approximately one-quarter of global marine capture landings are inflicted with illegal, unreported, and unregulated fishing activities (ISU Marine Programme, 2012).

NANOTECHNOLOGY FOR SUSTAINABLE AGRICULTURAL PRODUCTION

Nanotechnology has the potential to play an important role for sustainable agriculture, livestock, poultry, and fish production. It provides new agrochemical agents and new delivery mechanisms to improve crop productivity, and it has the potential to reduce pesticide use. Nanotechnology can augment the agricultural production, and its applications include nanoformulations of agrochemicals for applying pesticides and fertilizers for crop improvement; the application of nanosensors/nanobiosensors in crop protection for the identification of diseases and residues of agrochemicals; nanodevices for the genetic manipulation of plants; plant disease diagnostics; animal health, animal breeding, poultry production; and postharvest management. Precision farming techniques could be used to further improve crop yields sparing damage to soil and water, reducing nitrogen loss due to leaching and emissions, as well as enhancing long-term incorporation of nutrients by soil microorganisms. To name a few of the uses of nanotechnology are nanoparticle-mediated gene or DNA transfer in plants for the development of insect-resistant varieties, food processing and storage, nanofeed additives, and increased the shelf life of various products. Nanotechnology promises to accelerate the development of biomass-to-fuels production technologies (Sekhon, 2014). Nanoparticles could be industrially harvested, e.g., alfalfa plants grown in an AuCl_4^- -rich environment showed absorption of gold metal by the plants (Gardea-Torresdey, Parsons, & Gomez, 2002).

There are various applications of nanotechnology in animal husbandry. For example, improving the feeding efficiency and nutrition of farm animals, minimizing losses from animal diseases, and turning animal by-products and waste and environmental concerns into value-added products (Chen & Yada, 2011). Nanoparticles' use can produce poultry products at a much faster pace with high safety (Verma, Singh, & Vikas, 2012). The antimicrobial properties of nanobiotic-silver added in poultry nutrition combat microbial populations without developing microbial resistance. Furthermore, nanobiotic silver may increase anabolic activities. Gold nanoparticle-based diagnostic kits detect influenza virus in birds suffering from avian influenza (Emami, Madani, & Rezayat, 2012).

Nanotechnological applications in fish health include antibacterial surfaces in the aquaculture system, nanodelivery of veterinary products in fish food using porous nanostructures, and nanosensors for detecting pathogens in

the water (Handy, 2012; Rather, Sharma, & Aklakur, 2011). Nanoparticles have promise for improving protection of farmed fish against diseases caused by pathogens. Chitosan nanoparticles are reported to be promising carriers for an oral plasmid DNA vaccine (Sekhon, 2014).

Nanotechnology may serve high proteinous food to the vegetarians without slaughtering the animals, in the form of in vitro meat, cultured meat, or laboratory-grown meat. Nanotechnology may be the modern means in the quest to maintain nutritional quality while achieving the quantities needed to fill the stomachs of hungry people in the coming years. The transportability of livestock products with freshness is a great concern that may be ameliorated by the use of nanoparticles in the form of flexible pouches, laminates, and edible coatings (Verma et al., 2012).

Potential benefits of nanotechnology for agriculture, food, fisheries, and aquaculture are to be critically compared with the negative impact on soil, water, and environment and the occupational health of workers, if any. Feed and food ingredients standards, intelligent packaging, quick-detection systems of any toxicants, and affordable cost should be taken into account in the food chain so as to increase the consumer acceptance. Safety measures and strict regulation of the nanomaterials are to be implemented so that there should not be any detrimental effect on human and animal health as well as on environment.

SOCIAL SECURITY

Global production of sufficient food does not ensure balanced food for all. There are many complex social issues like access to technology and distribution of food, purchasing power of the poor which are to be addressed. Resource-poor small farmers should have access to biotechnological tools and products. Policy matter and IPR regimes should not be a hindrance to avail technological know-how in developing countries. There should be a policy to enable the producers to use sustainable practices to market their goods. More climate finance is needed to fund the developing countries' action on climate change. Sustainable agriculture, animal husbandry, fisheries, and forestry activities are to be funded by climate finance for the large scale transformation and the development of climate-smart food production system.

Food Waste

Wastage of food is a great concern to global community. You will be surprised to know the WHO data on food wastage. According to a rough estimate, about one-third of the food farm produces ends up discarded, spoiled, or eaten by pests which are roughly equivalent to 1.3 billion tons of edible food. The food lost calculated in the tune of USD 1 trillion in economic costs, around USD 700 billion in environmental costs, and around USD 900

billion in social cost. In United States, food waste accounts to \$218 billion equivalent to 1.3% of its GDP. If we save one-fourth of the food currently lost or wasted, we can feed 870 billion poor. Food lost and waste is responsible for about 8% of global greenhouse gas emissions.

CONCLUSION

In one way, agriculture provides human food and well-being whereas in other way, it is the largest single cause of global warming and loss of ecosystem. We have to increase the food production double to feed the growing population on this planet without exhausting the global resources and keeping the planet cool and habitable. This can be achieved successfully by sustainable food production with five key approaches viz. stop farmland expansion, close yield gap, strategic use of inputs, shift diets, and reduce food waste. Regulation of deforestation, promoting social forestry and ecotourism, can provide environmental benefits without affecting agricultural production or economic wellbeing. By adopting better management and improved genetics and by minimizing the yield gaps through improved use of existing crop varieties, current food production could be increased by nearly 60%. Water and nutrients management should be done in more intelligent ways. This will ensure that we can grow more food, but with less harm to the environment. Allied agricultural resources like animal husbandry, fishery, forestry, and apiculture should be explored to achieve global food security. Avoid growing animal feed or nonfood crops, biofuels on top croplands. Focusing human food production on croplands may enhance food supply nearly 50%. Cultivation of animal feed or biofuels production away from prime cropland boost food availability for human consumption. One-third of the food produced in farms go waste due to spoilage, discard, or taken by pests. By preventing the waste in the process from farm to mouth could make food available for consumption to another 50% population. Nanotechnology has the potential to play an important role for sustainable agriculture, livestock, poultry, and fish production. Global production of sufficient food does not ensure balanced food for all. There are many complex social issues like access to technology and distribution of food, purchasing power of the poor which are to be addressed.

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Biotechnology for Sustainable Agriculture

Emerging Approaches and Strategies

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Biotechnology for Sustainable Agriculture is an outstanding collection of current research that integrates basic and advanced concepts of agricultural biotechnology with future development prospects. Using biotechnology with sustainable agriculture effectively contributes to increased agricultural productivity, enhanced food security, poverty reduction, and more ecologically sustainable livestock production.

Written by a panel of experts, *Biotechnology for Sustainable Agriculture* enables the development of solutions by providing foundational information on current innovative biotechnologies, such as recombinant DNA technology and genetic engineering, which have tremendous potential for positively impacting food production and environmental protection. This unique single resource provides coverage of the broad range of highly relevant biotechnology topics, as well as highlighting climate change impacts on agriculture, livestock production, and aquaculture and the strategies for mitigation.

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- Explores important facets including food security, climate change, microbial biotechnology, as well as current innovative biotechnologies in climate-smart sustainable agriculture and livestock production
- Includes latest nanotechnology information, as well as emerging and cutting-edge technologies
- Presents crucial information for scientific researchers developing further technologies

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